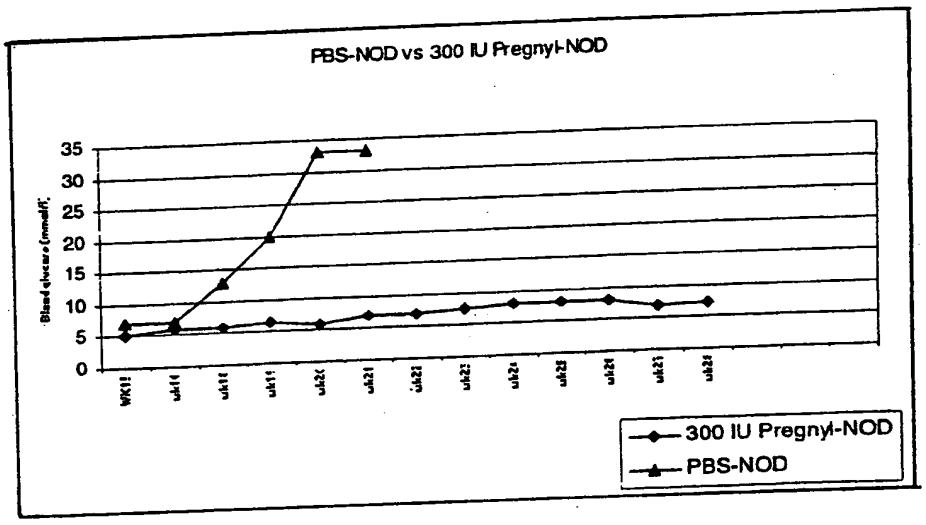

PBS-NOD vs 300 IU Pregnyl-NOD
35
30
25
20
15
10
5
0
300 IU Pregnyl-NOD
PBS-NOD

*Figure 1.*

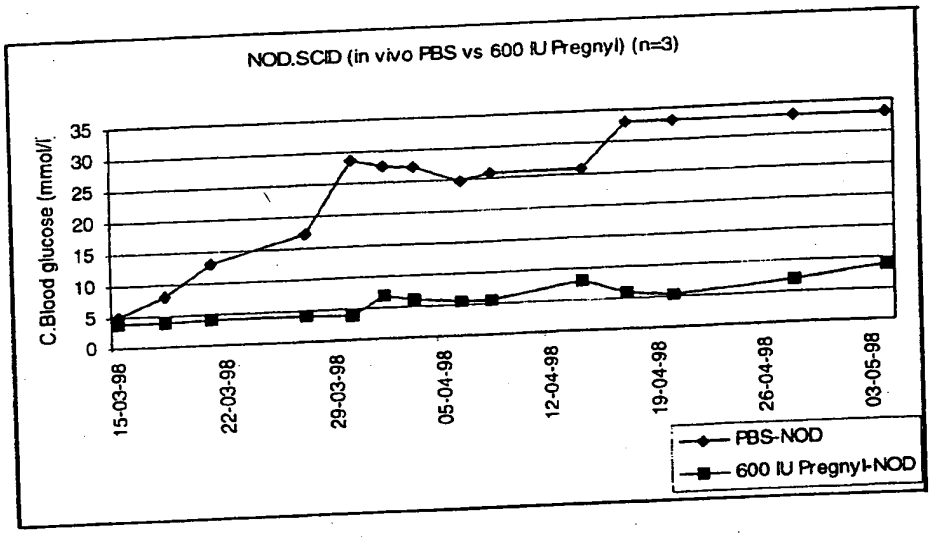

NOD.SCID (in vivo PBS vs 600 IU Pregnyl) (n=3)
C.Blood glucose (mmol/l)
35
30
25
20
15
10
5
0
15-03-98
22-03-98
29-03-98
05-04-98
12-04-98
19-04-98
26-04-98
03-05-98
PBS-NOD
600 IU Pregnyl-NOD

*Figure 2.*

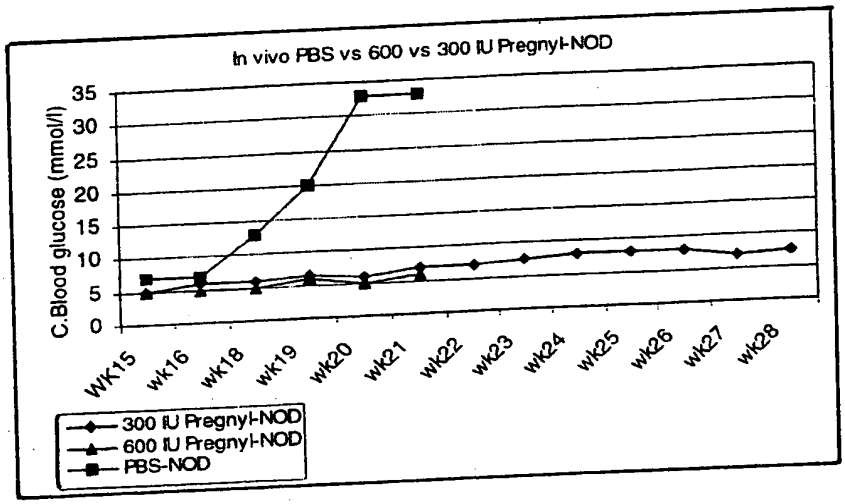

In vivo PBS vs 600 vs 300 IU Pregnyl-NOD
C.Blood glucose (mmol/l)
35
30
25
20
15
10
5
0
WK15
wk16
wk18
wk19
wk20
wk21
wk22
wk23
wk24
wk25
wk26
wk27
wk28
300 IU Pregnyl-NOD
600 IU Pregnyl-NOD
PBS-NOD

*Figure 3.*

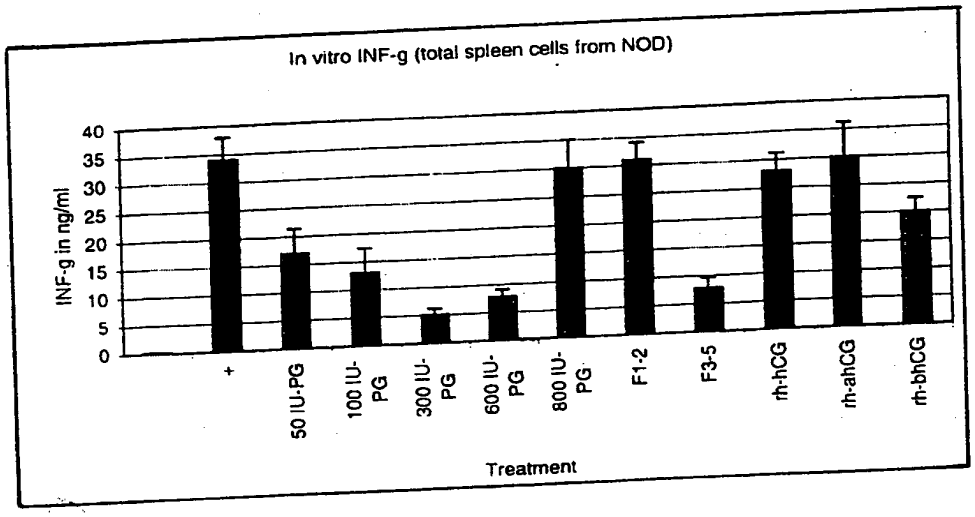

In vitro INF-g (total spleen cells from NOD)
INF-g in ng/ml
40
35
30
25
20
15
10
5
0
+
50 IU-PG
100 IU-PG
300 IU-PG
600 IU-PG
800 IU-PG
F1-2
F3-5
rh-hCG
rh-ahCG
rh-bhCG
Treatment

*Figure 4.*

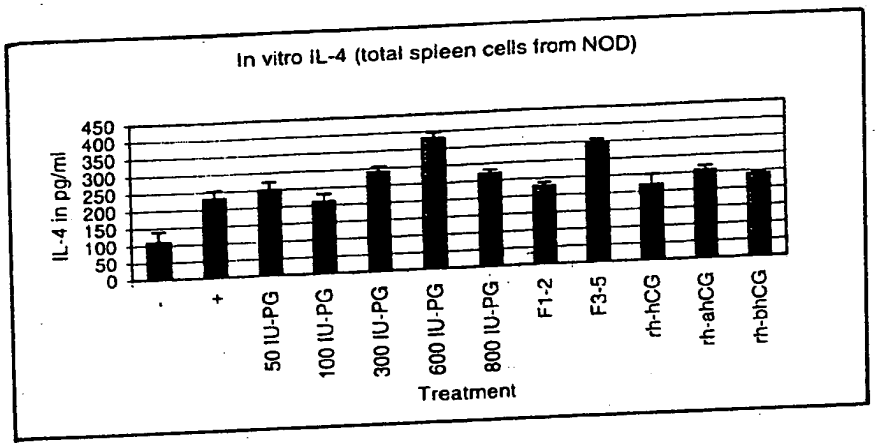

In vitro IL-4 (total spleen cells from NOD)
IL-4 in pg/ml
450
400
350
300
250
200
150
100
50
0
-
+
50 IU-PG
100 IU-PG
300 IU-PG
600 IU-PG
800 IU-PG
F1-2
F3-5
rh-hCG
rh-ahCG
rh-bhCG
Treatment

*Figure 5.*

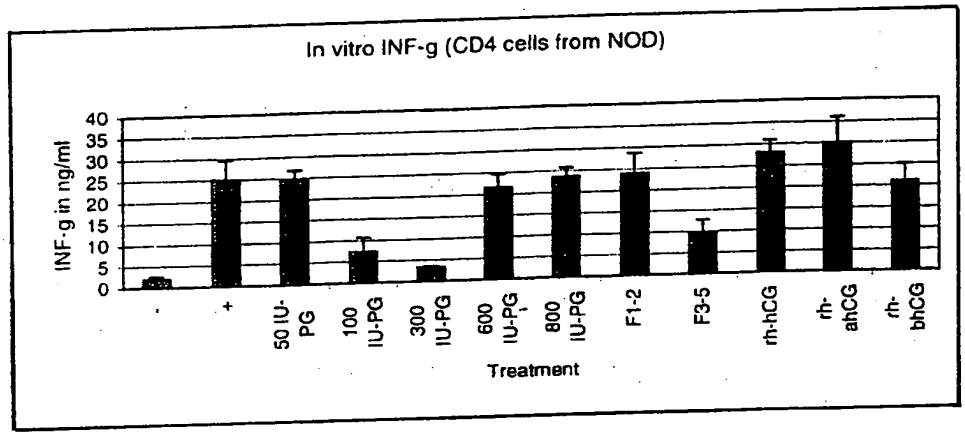

In vitro INF-g (CD4 cells from NOD)
INF-g in ng/ml
40
35
30
25
20
15
10
5
0
-
+
50 IU-PG
100 IU-PG
300 IU-PG
600 IU-PG
800 IU-PG
F1-2
F3-5
rh-hCG
rh-ahCG
rh-bhCG
Treatment

*Figure 6.*

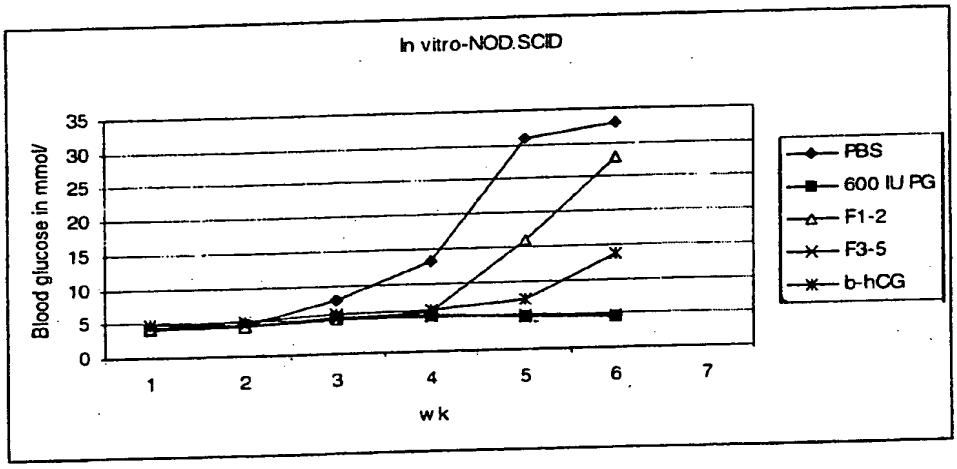

In vitro-NOD.SCID
Blood glucose in mmol/
35
30
25
20
15
10
5
0
1
2
3
4
5
6
7
wk
PBS
600 IU PG
F1-2
F3-5
b-hCG

*Figure 7.*

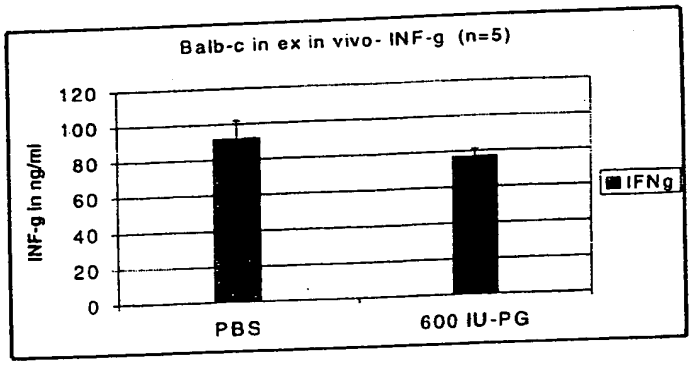



*Figure 8.*

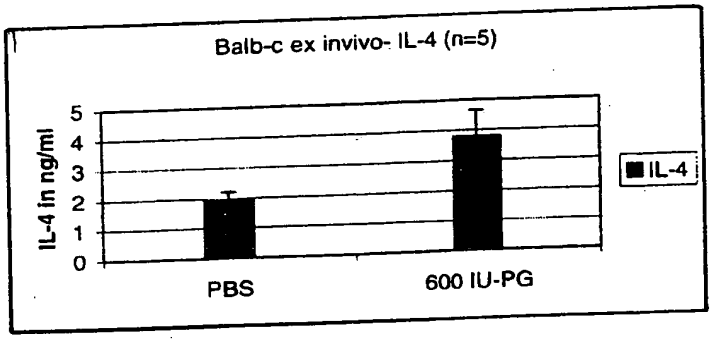



*Figure 9.*

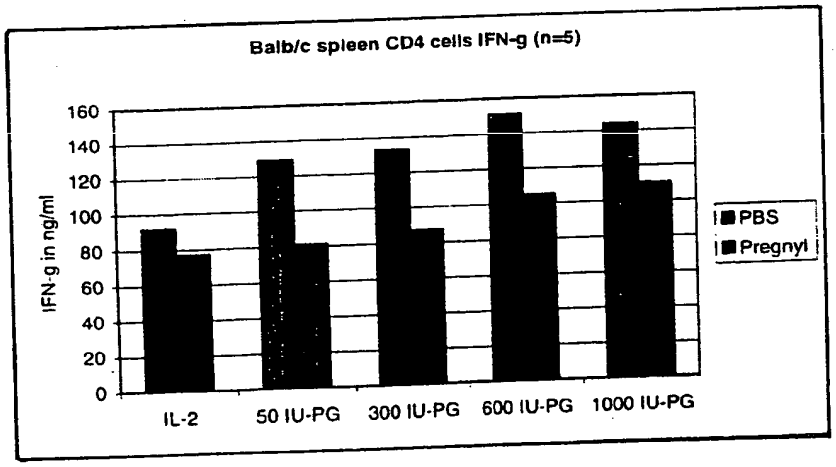



*Figure 10.*

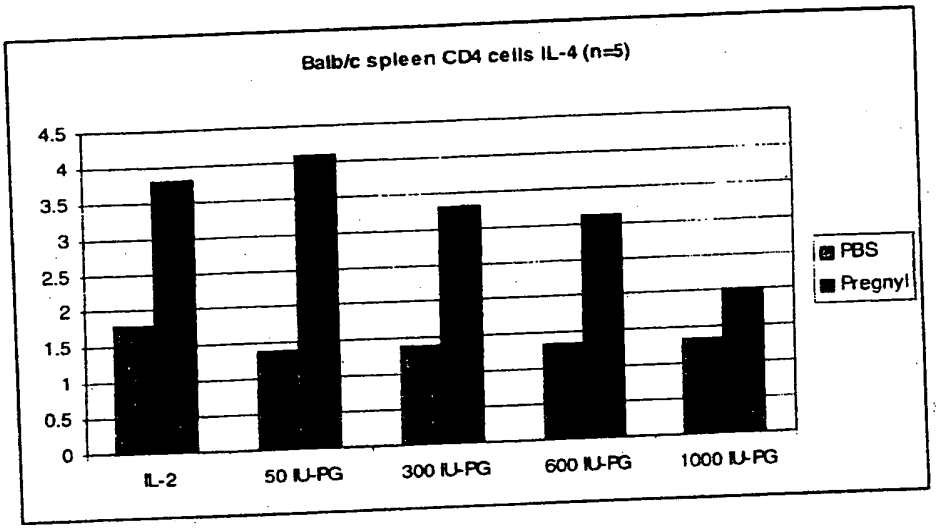

Balb/c spleen CD4 cells IL-4 (n=5)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
IL-2
50 IU-PG
300 IU-PG
600 IU-PG
1000 IU-PG
PBS
Pregnyl

*Figure 11.*

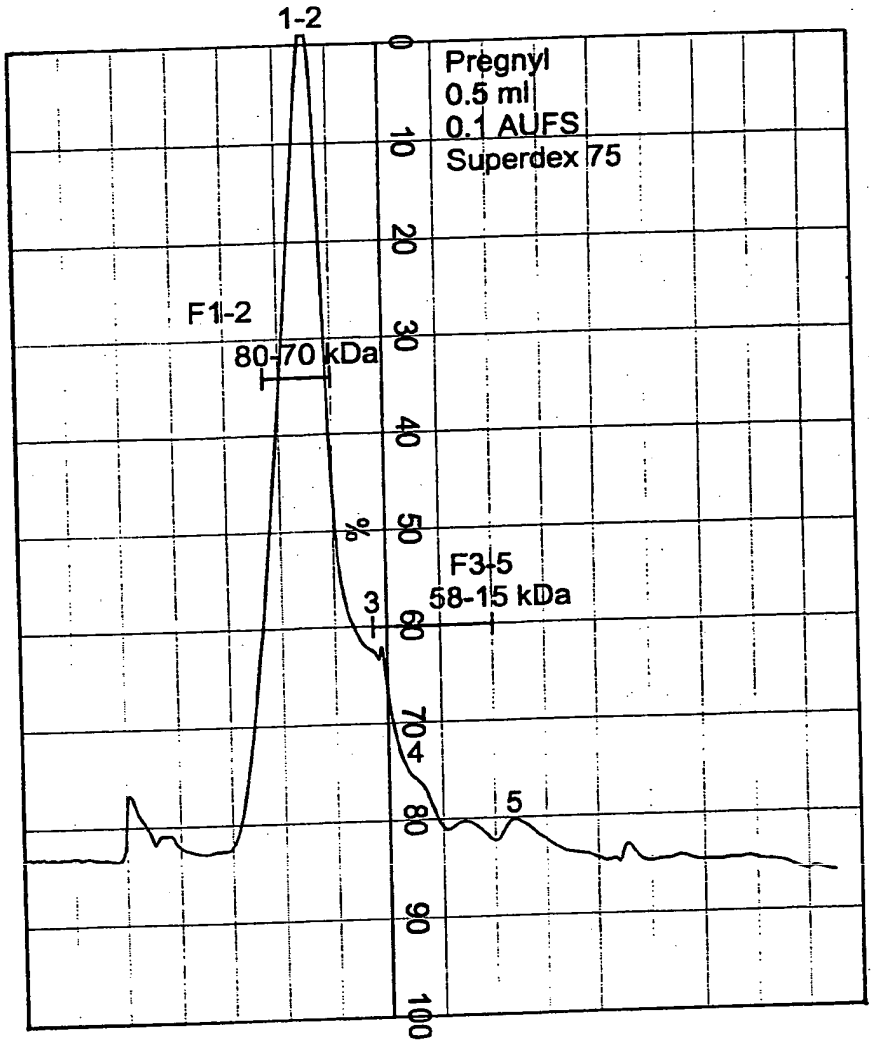

1-2
Pregnyl
0.5 ml
0.1 AUFS
Superdex 75
F1-2
80-70 kDa
%
F3-5
58-15 kDa
3
4
5
0
10
20
30
40
50
60
70
80
90
100

Figure 12

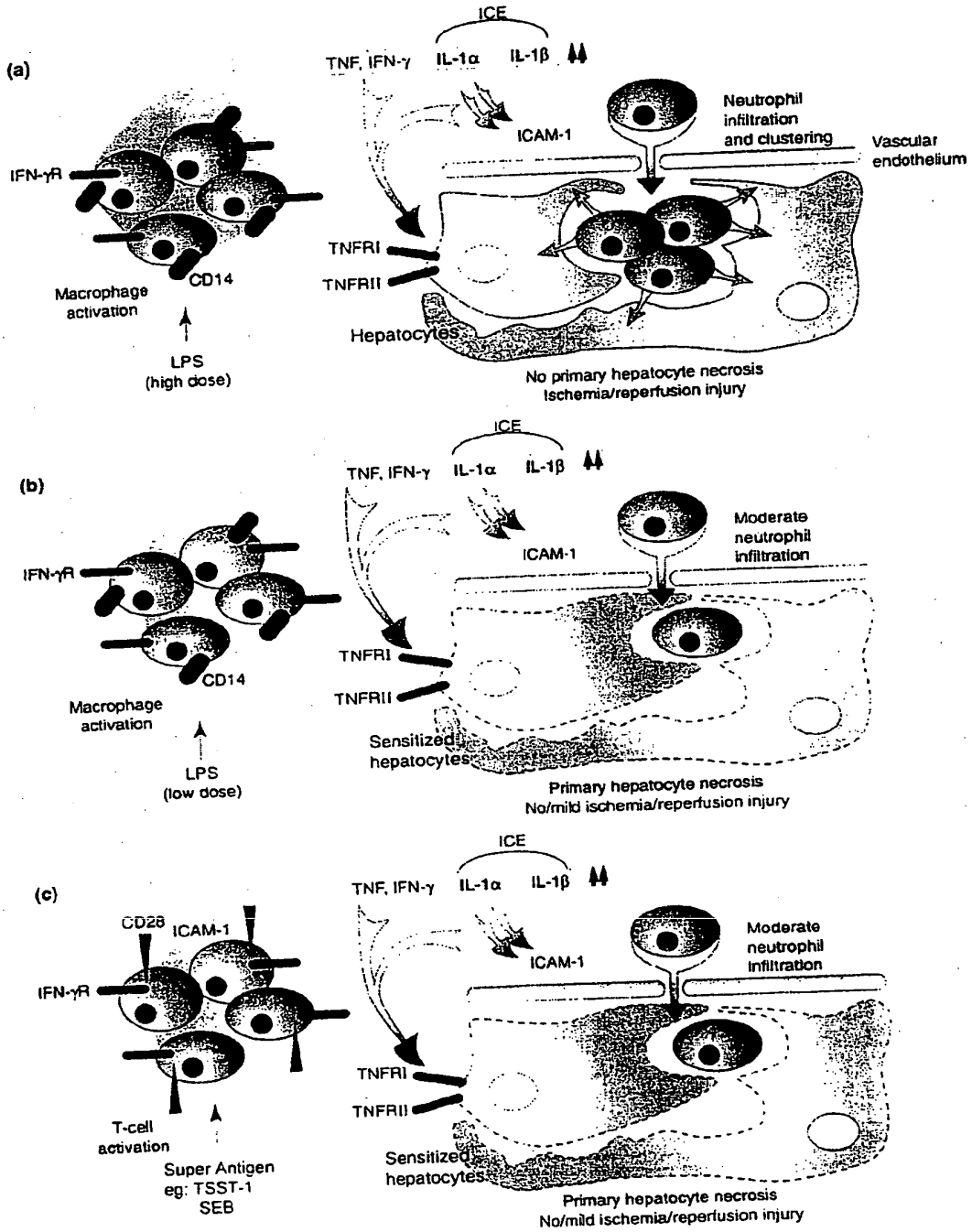

(a)
ICE
TNF, IFN-γ
IL-1α
IL-1β
ICAM-1
Neutrophil infiltration and clustering
Vascular endothelium
IFN-γR
CD14
Macrophage activation
LPS (high dose)
TNFRI
TNFRII
Hepatocytes
No primary hepatocyte necrosis Ischemia/reperfusion injury
(b)
ICE
TNF, IFN-γ
IL-1α
IL-1β
ICAM-1
Moderate neutrophil infiltration
IFN-γR
CD14
Macrophage activation
LPS (low dose)
TNFRI
TNFRII
Sensitized hepatocytes
Primary hepatocyte necrosis No/mild ischemia/reperfusion injury
(c)
ICE
TNF, IFN-γ
IL-1α
IL-1β
CD28
ICAM-1
ICAM-1
Moderate neutrophil infiltration
IFN-γR
T-cell activation
Super Antigen eg: TSST-1 SEB
TNFRI
TNFRII
Sensitized hepatocytes
Primary hepatocyte necrosis No/mild ischemia/reperfusion injury

Figure 13

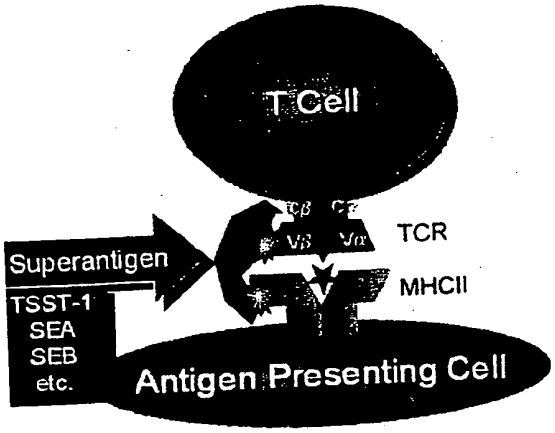

T Cell
Cβ
Cα
Vβ
Vα
TCR
MHCII
Superantigen
TSST-1
SEA
SEB
etc.
Antigen Presenting Cell

Figure 14

Figure 15

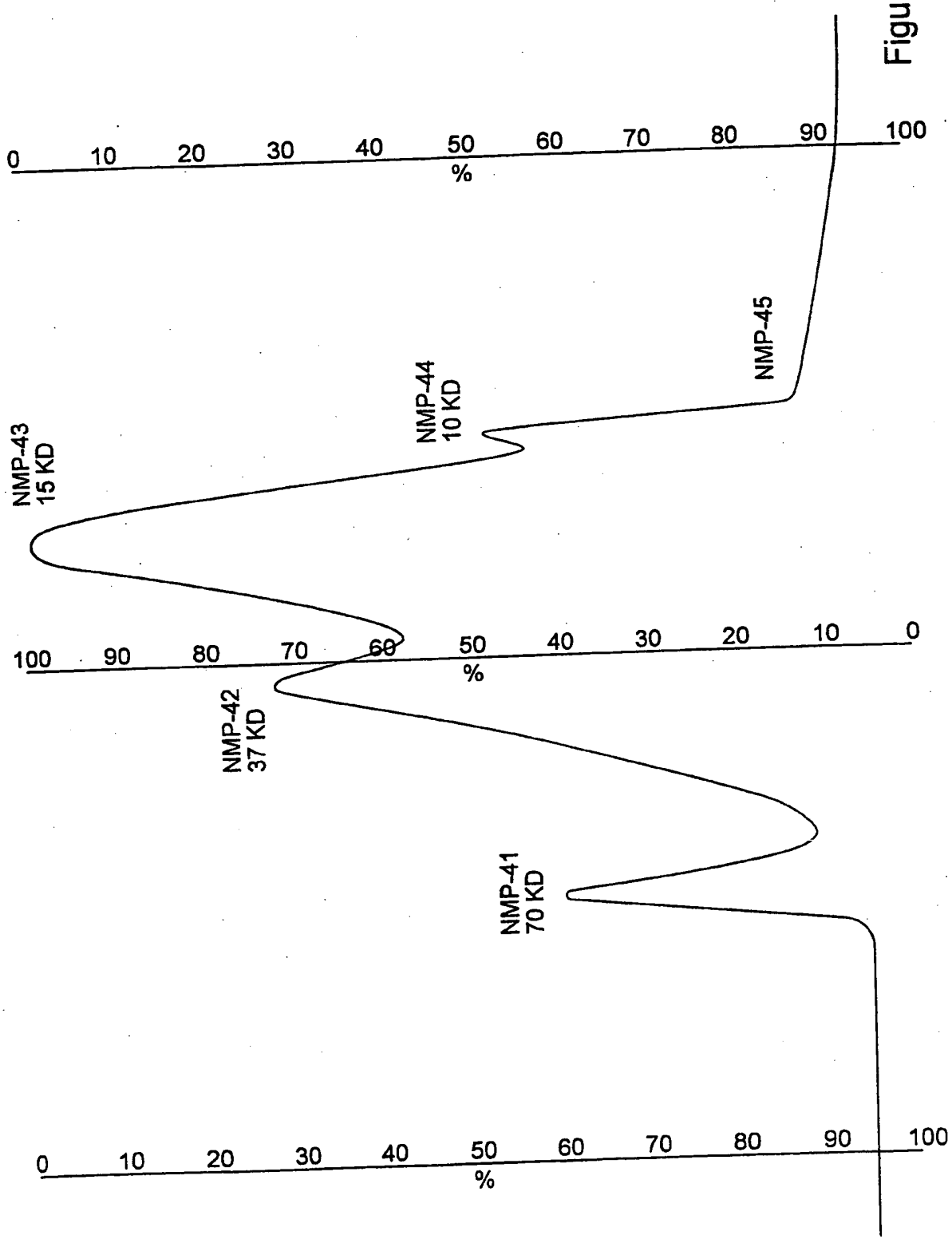

0 10 20 30 40 50 60 70 80 90 100
%
NMP-45
NMP-44
10 KD
NMP-43
15 KD
100 90 80 70 60 50 40 30 20 10 0
%
NMP-42
37 KD
NMP-41
70 KD
0 10 20 30 40 50 60 70 80 90 100
%

Figure 16

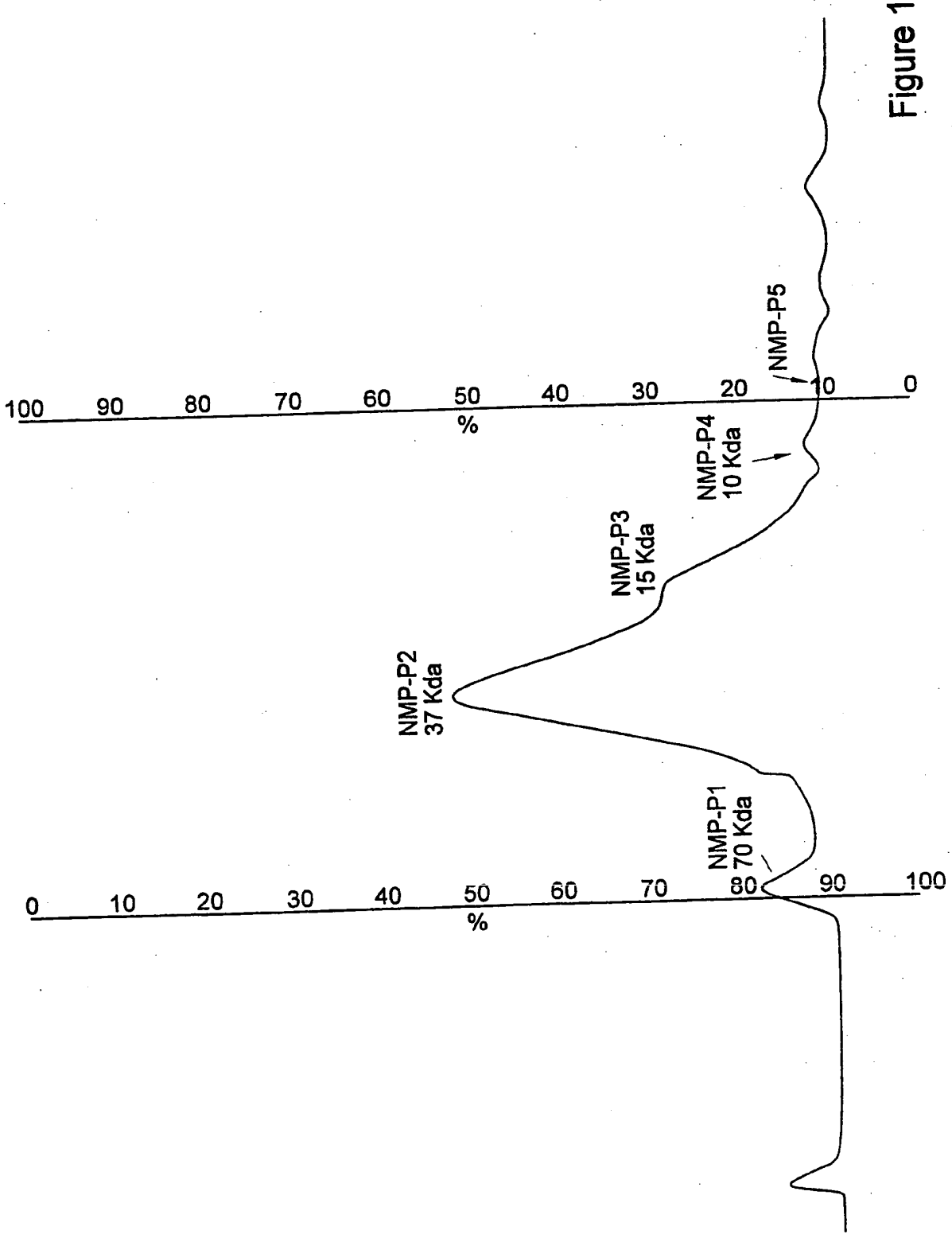

NMP-P5
100 90 80 70 60 50 40 30 20 10 0
%
NMP-P4
10 Kda
NMP-P3
15 Kda
NMP-P2
37 Kda
NMP-P1
70 Kda
0 10 20 30 40 50 60 70 80 90 100
%

Figure 17

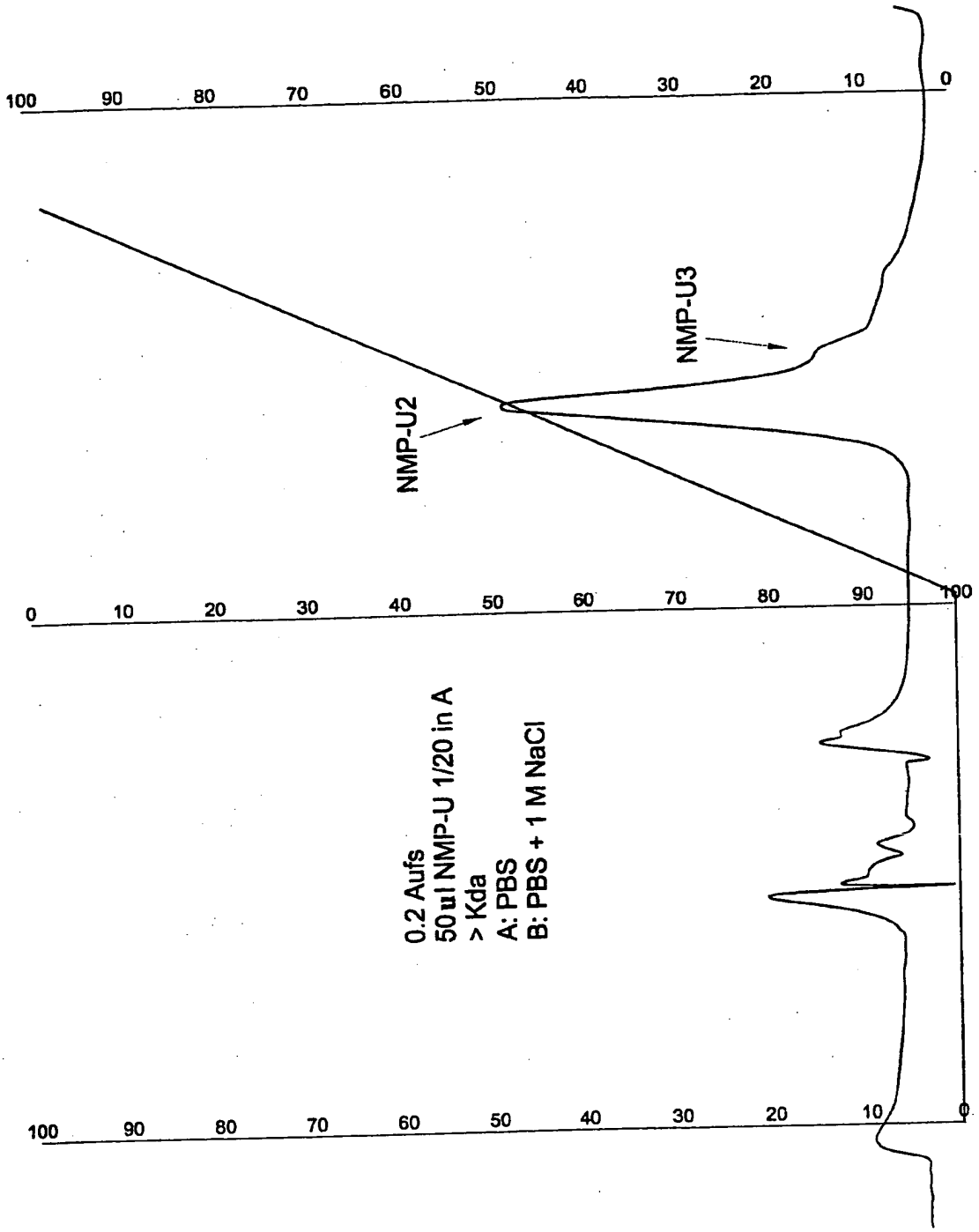

100 90 80 70 60 50 40 30 20 10 0
NMP-U3
NMP-U2
0 10 20 30 40 50 60 70 80 90 100
0.2 Aufs
50 ul NMP-U 1/20 in A
> Kda
A: PBS
B: PBS + 1 M NaCl
100 90 80 70 60 50 40 30 20 10 0

Figure 18

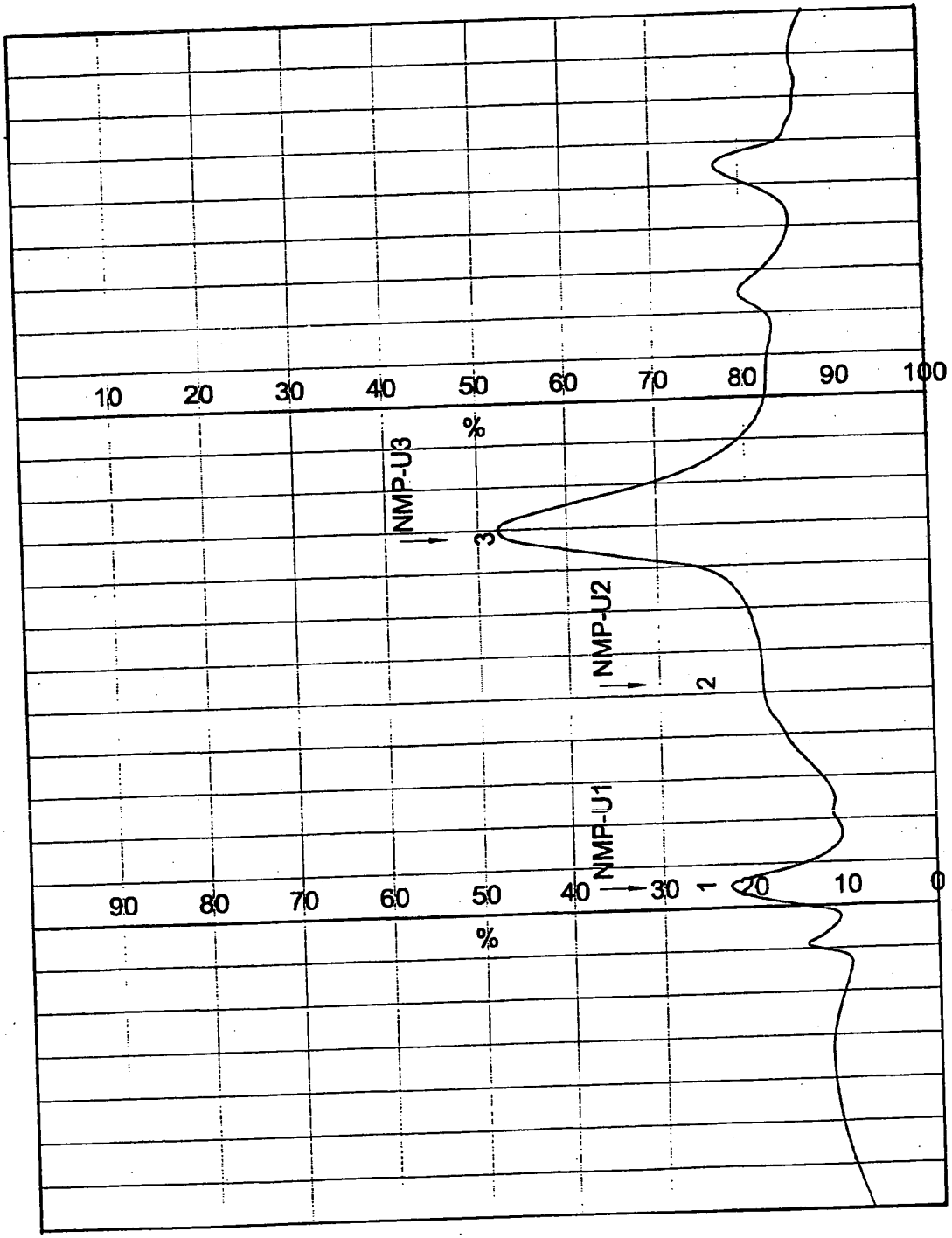
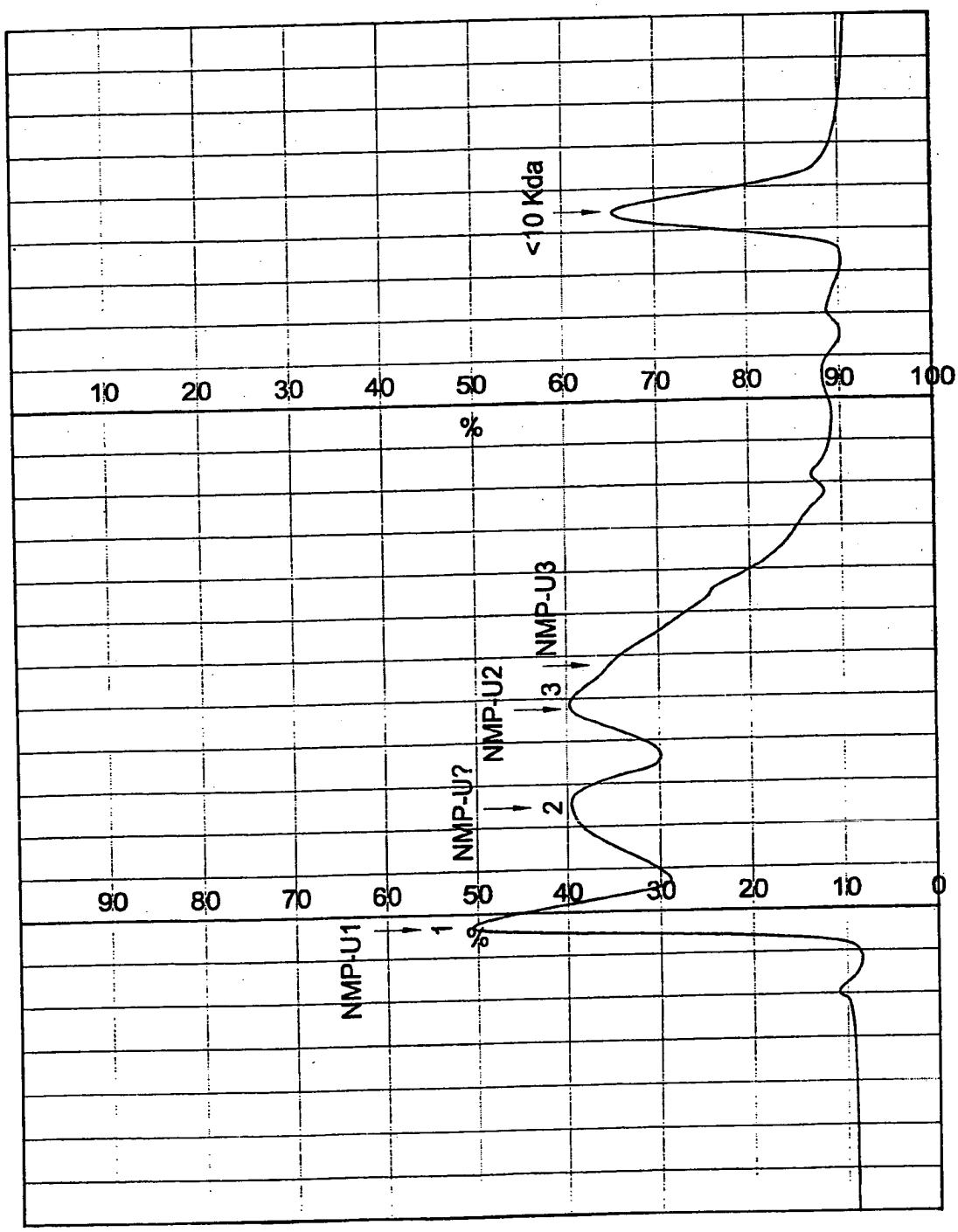

10
20
30
40
50
60
70
80
90
100
%
NMP-U3
3
NMP-U2
2
NMP-U1
1
90
80
70
60
50
40
30
20
10
0
%

Figure 19


<10 Kda
10
20
30
40
50
60
70
80
90
100
%
NMP-U3
3
NMP-U2
NMP-U?
2
90
80
70
60
50
40
30
20
10
0
%
NMP-U1
1

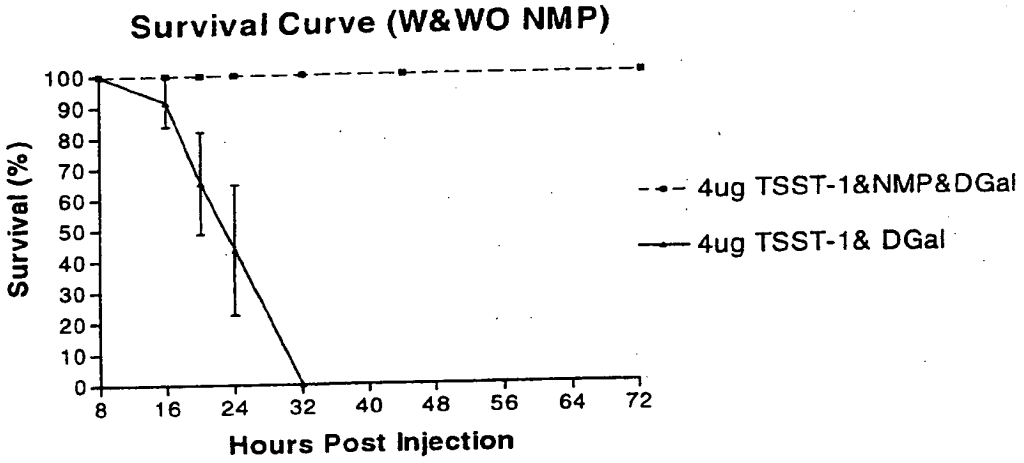

Survival Curve (W&WO NMP)
Survival (%)
100
90
80
70
60
50
40
30
20
10
0
8
16
24
32
40
48
56
64
72
Hours Post Injection
4ug TSST-1&NMP&DGal
4ug TSST-1& DGal

Figure 20

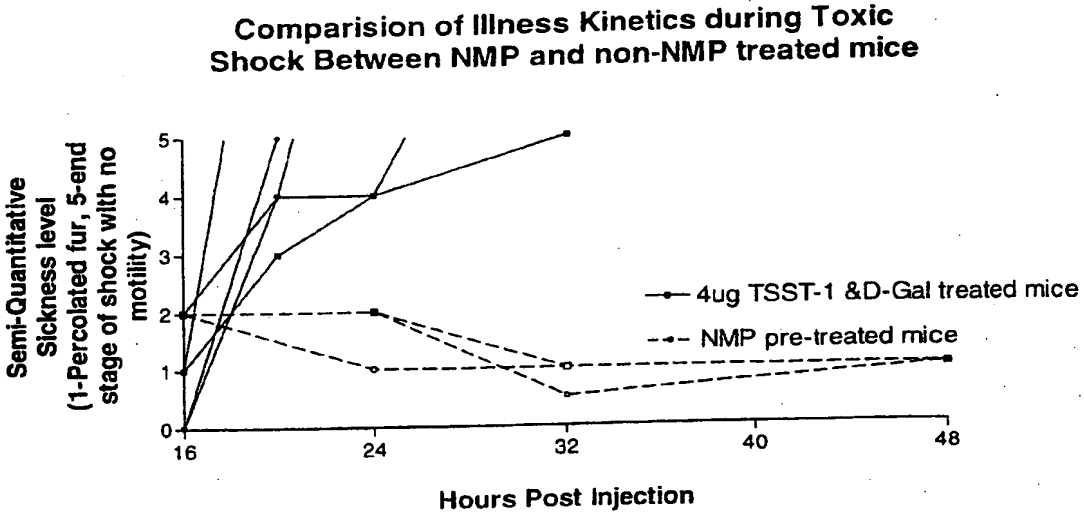

Comparision of Illness Kinetics during Toxic Shock Between NMP and non-NMP treated mice
Semi-Quantitative Sickness level (1-Percolated fur, 5-end stage of shock with no motility)
0
1
2
3
4
5
16
24
32
40
48
Hours Post Injection
4ug TSST-1 &D-Gal treated mice
NMP pre-treated mice

Figure 21

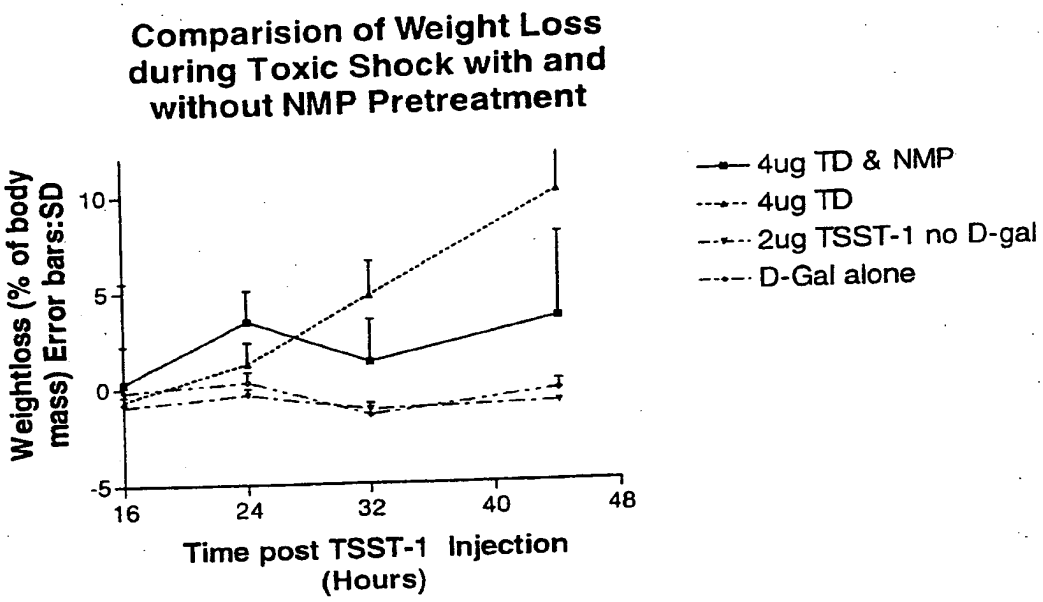

Comparision of Weight Loss during Toxic Shock with and without NMP Pretreatment
Weightloss (% of body mass) Error bars:SD
10
5
0
-5
16
24
32
40
48
Time post TSST-1 Injection (Hours)
4ug TD & NMP
4ug TD
2ug TSST-1 no D-gal
D-Gal alone

Figure 22

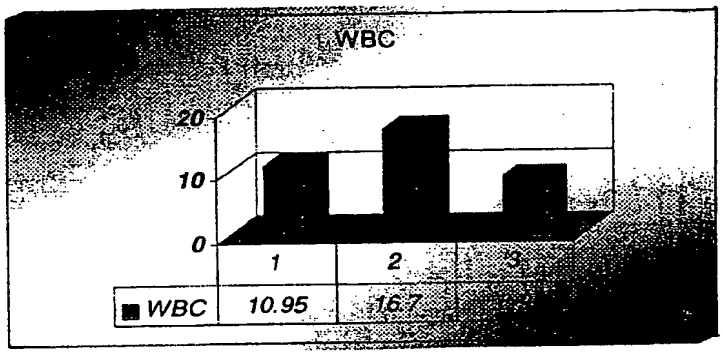



Figure 23

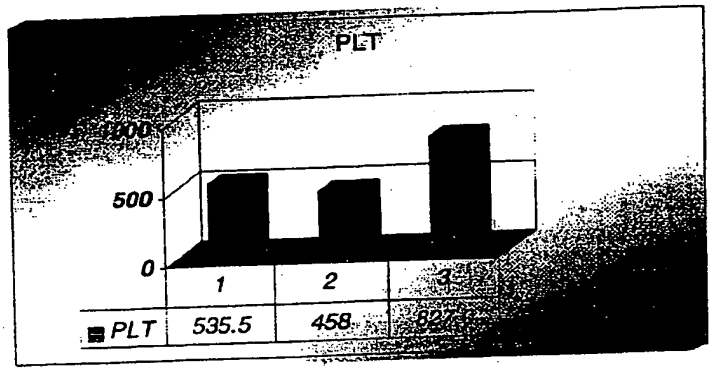



Figure 24

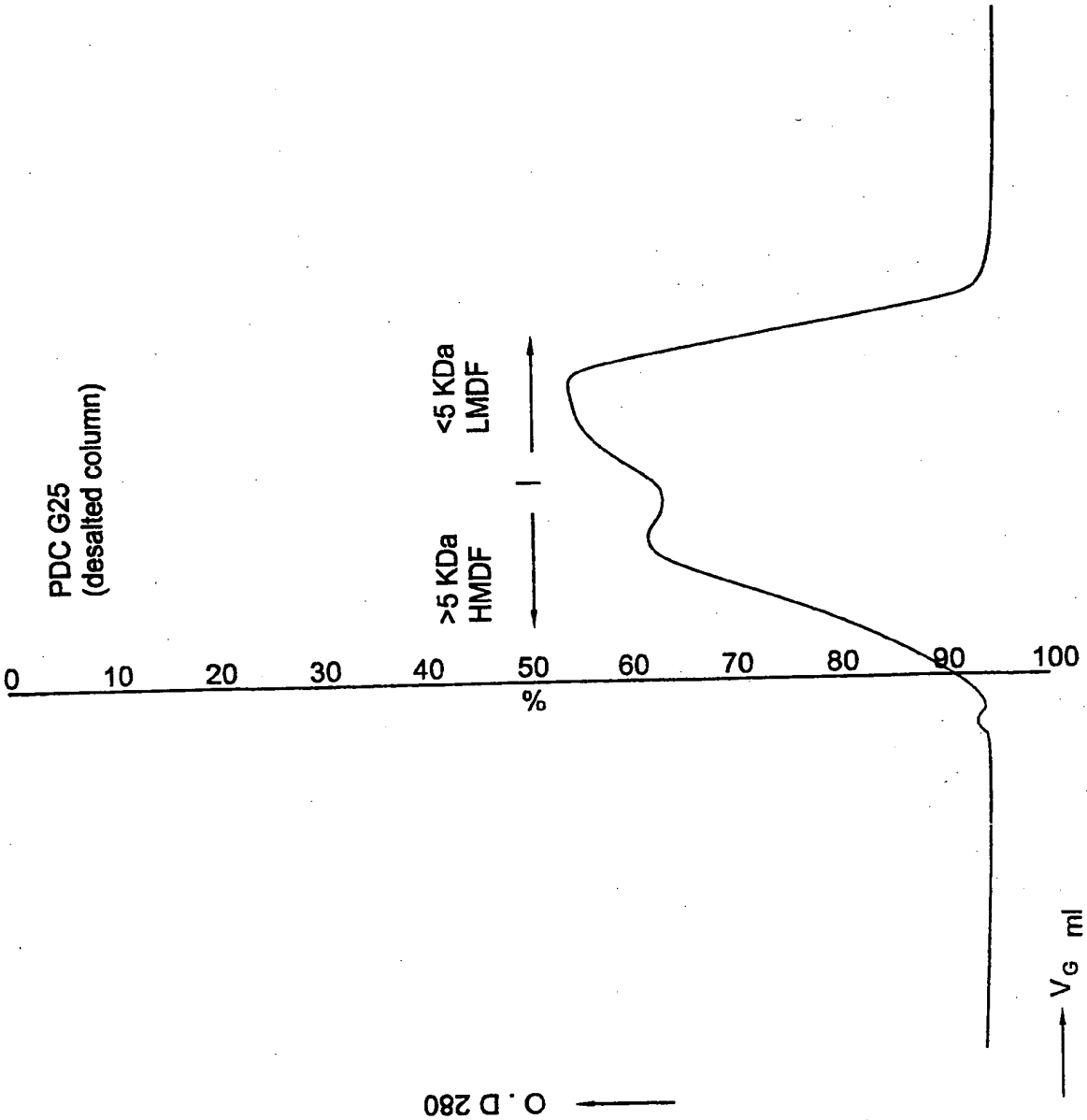
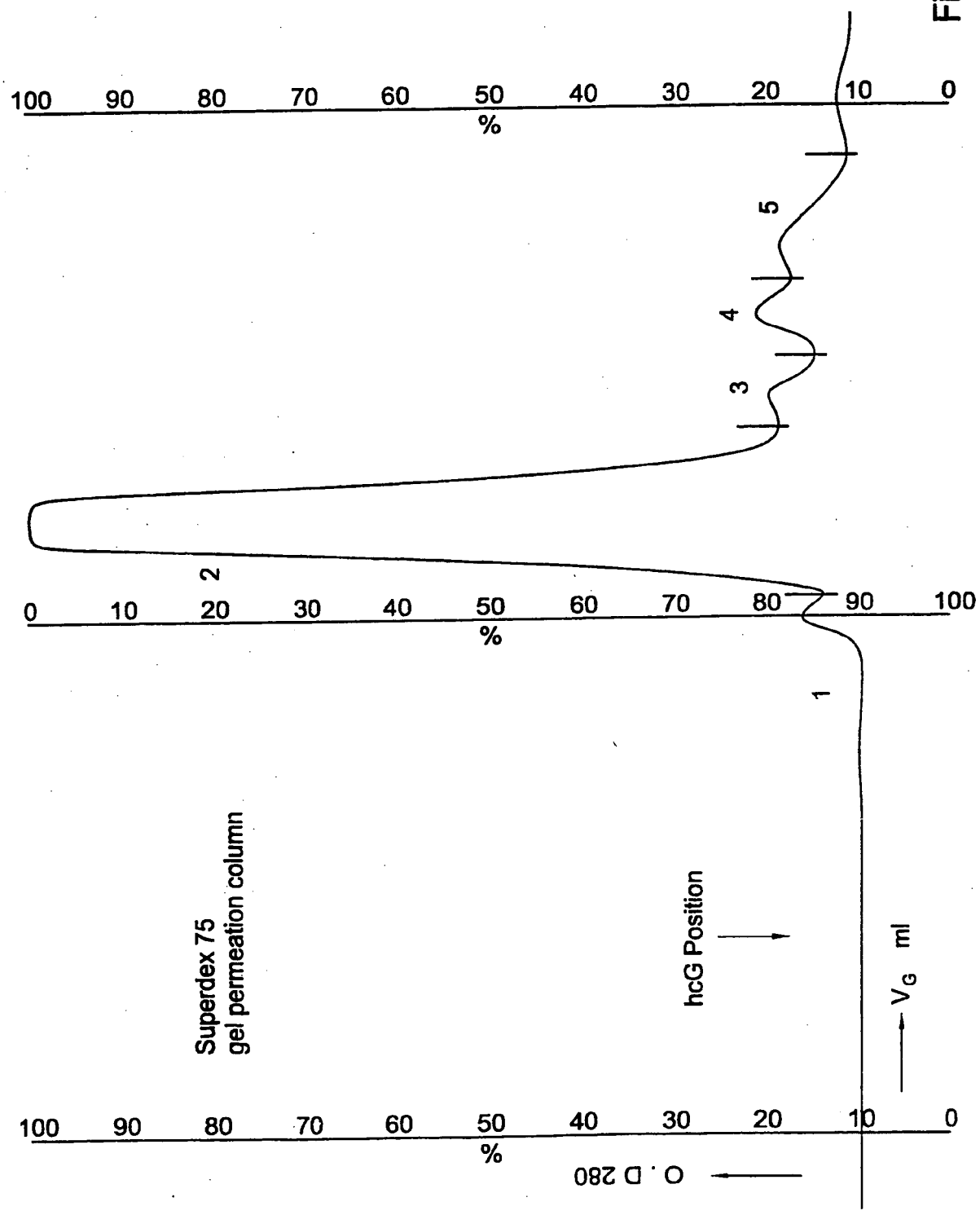

PDC G25
(desalted column)
<5 KDa
LMDF
>5 KDa
HMDF
0
10
20
30
40
50
60
70
80
90
100
%
O . D 280
$V_G$ ml

Figure 25

Figure 26


100 90 80 70 60 50 40 30 20 10 0
%
5
4
3
2
0 10 20 30 40 50 60 70 80 90 100
%
1
Superdex 75
gel permeation column
hcG Position
$V_G$ ml
100 90 80 70 60 50 40 30 20 10 0
%
O . D 280

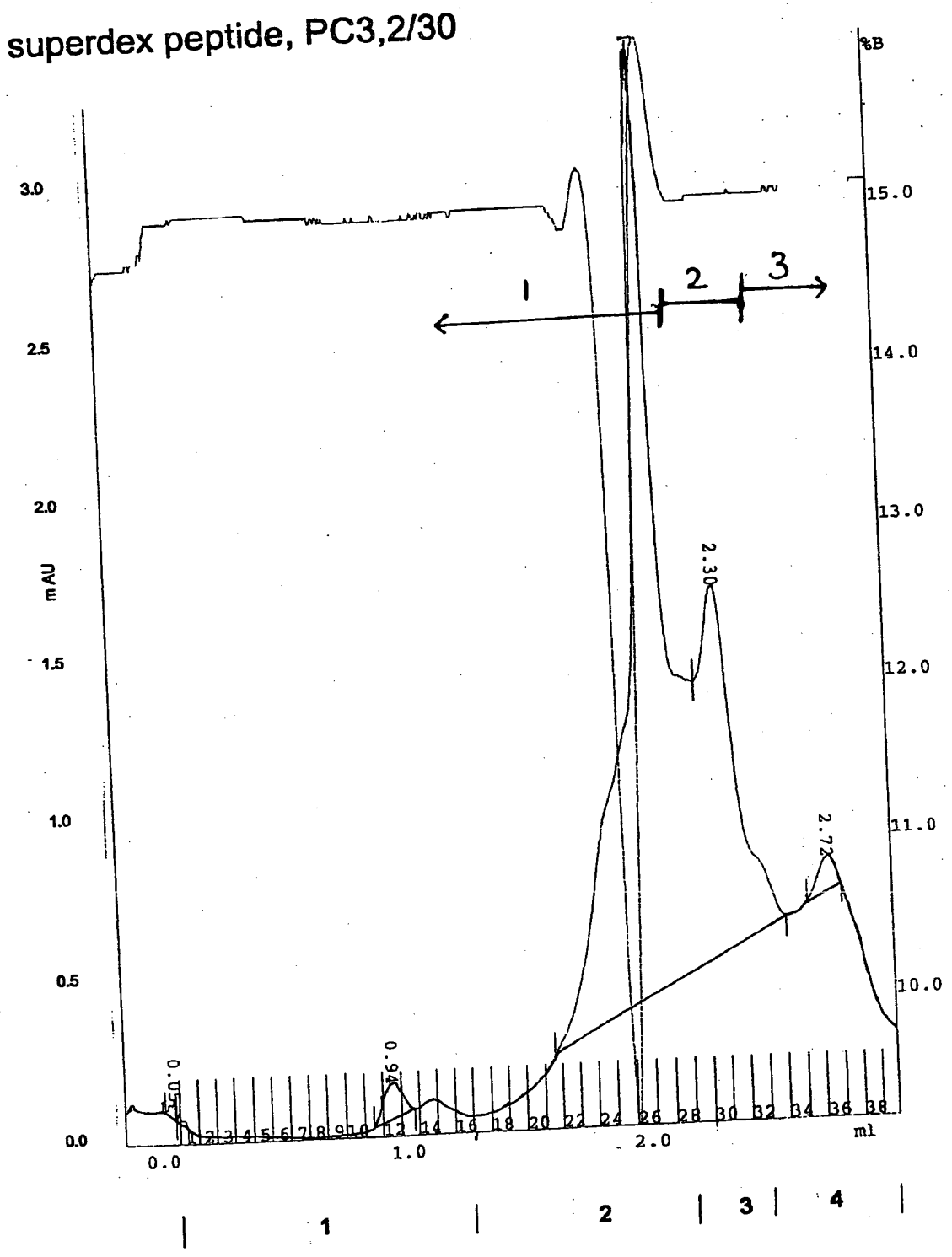

superdex peptide, PC3,2/30
%B
mAU
3.0
2.5
2.0
1.5
1.0
0.5
0.0
15.0
14.0
13.0
12.0
11.0
10.0
1
2
3
2.30
2.72
0.96
0.0
1.0
2.0
ml
1
2
3
4

Figure 27

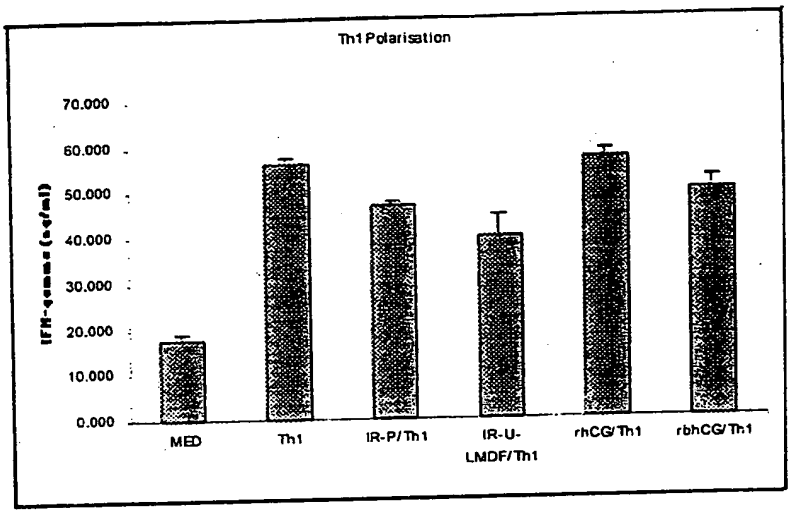



Figure 28.

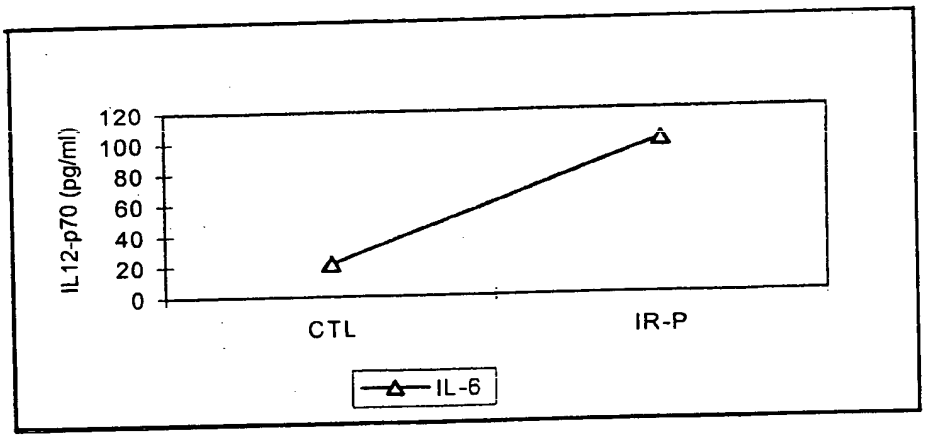
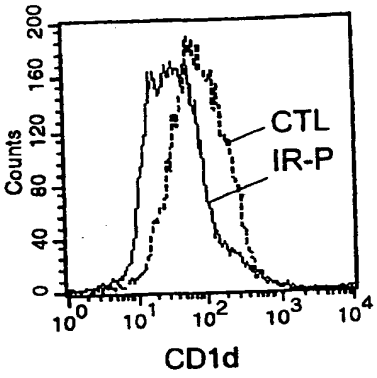
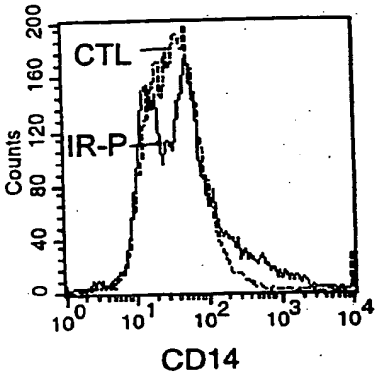
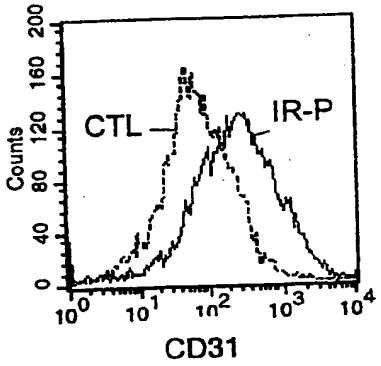


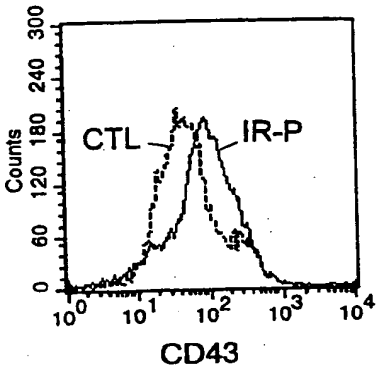

figure 45

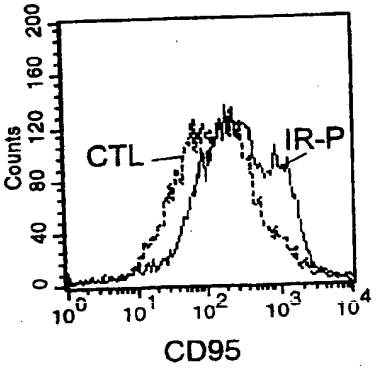
Figure 29A

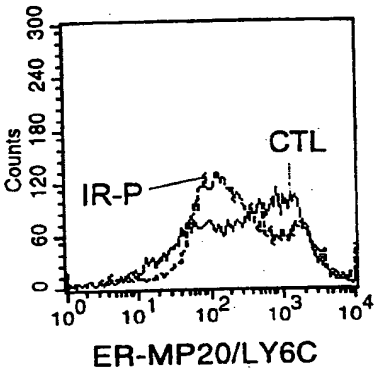

CTL
IR-P
Counts
CD1d

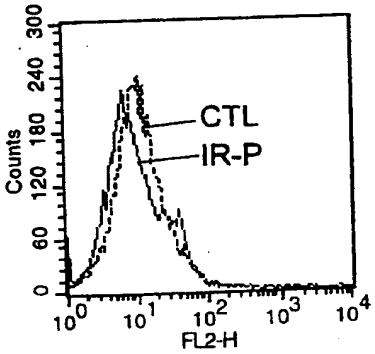

CTL
IR-P
Counts
CD14

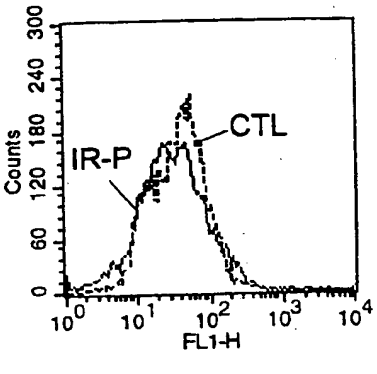

CTL
IR-P
Counts
CD31


CTL
IR-P
Counts
CD43

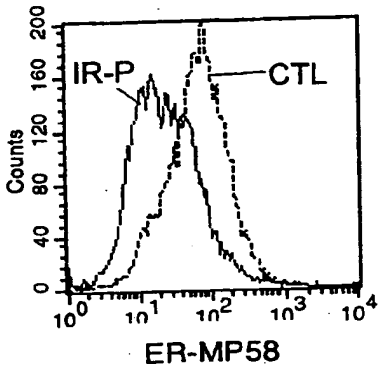

CTL
IR-P
Counts
CD95

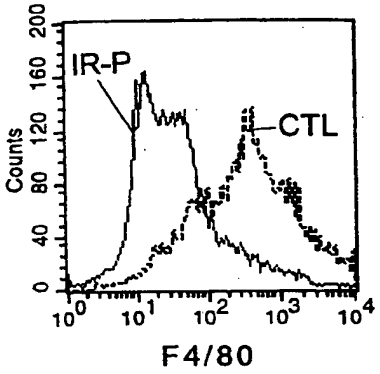

CTL
IR-P
Counts
ER-MP20/LY6C

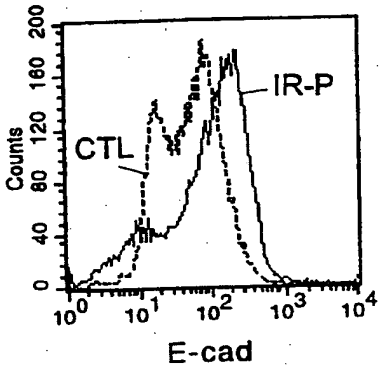

CTL
IR-P
Counts
FL2-H
NEG. CONTR.

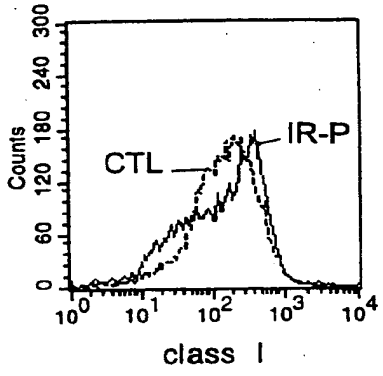

CTL
IR-P
Counts
FL1-H
NEG. CONTR.

# Figure 29B


IR-P
CTL
Counts
ER-MP58


IR-P
CTL
Counts
F4/80


IR-P
CTL
Counts
E-cad


IR-P
CTL
Counts
class I

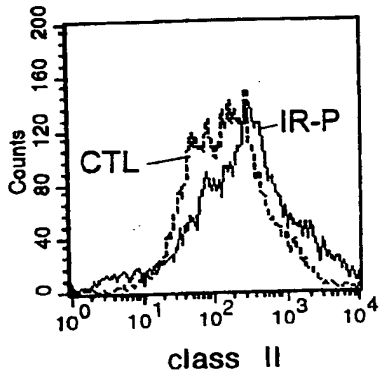
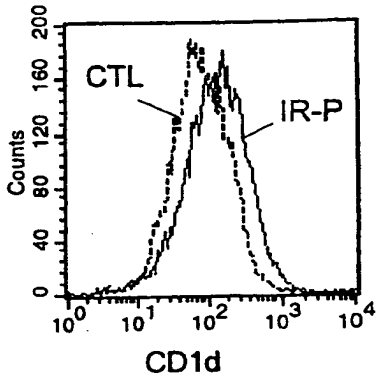
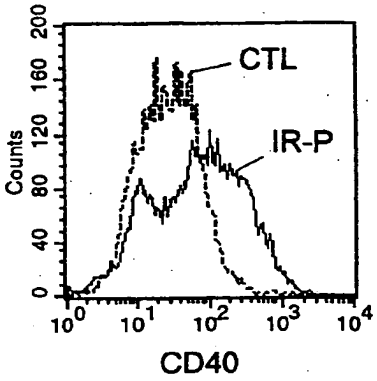
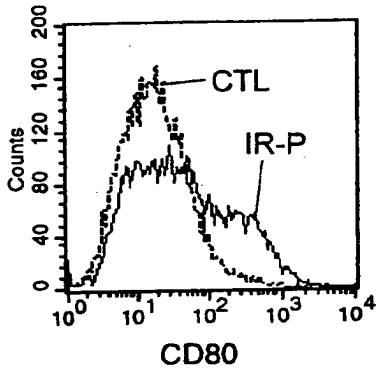
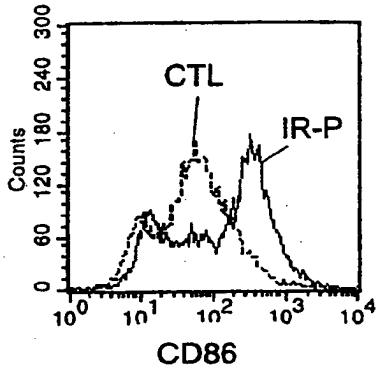

IR-P
CTL
Counts
class II

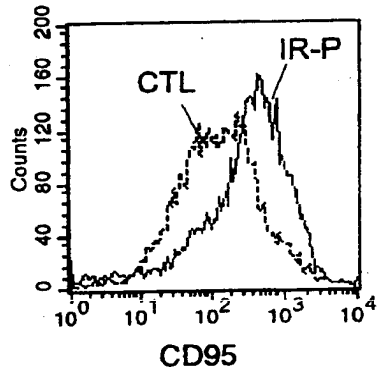
Figure 30A

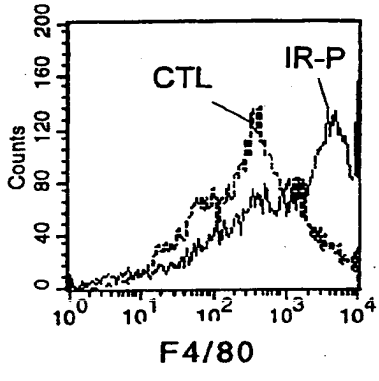

CTL
IR-P
Counts
CD1d

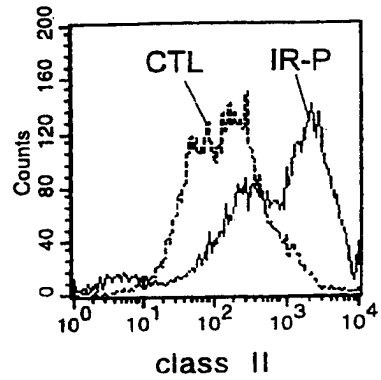

CTL
IR-P
Counts
CD40

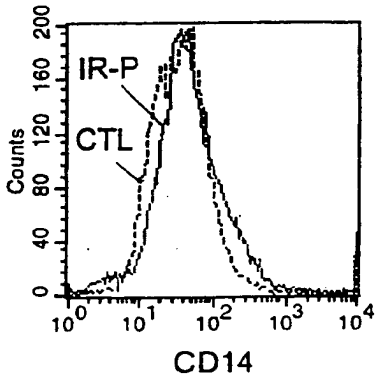

CTL
IR-P
Counts
CD80


CTL
IR-P
Counts
CD86

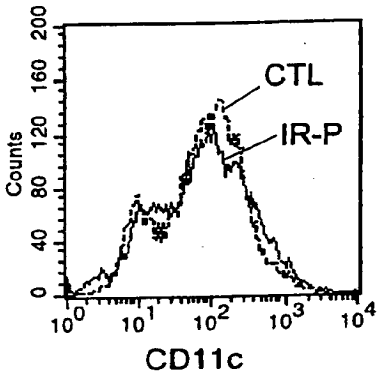

IR-P
CTL
Counts
CD95


IR-P
CTL
Counts
F4/80

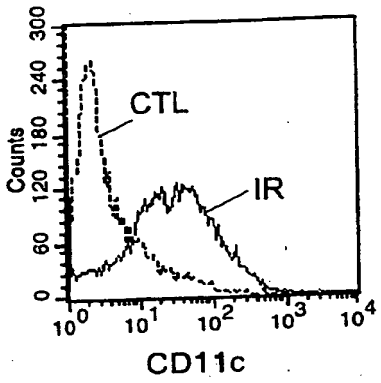

CTL
IR-P
Counts
class II

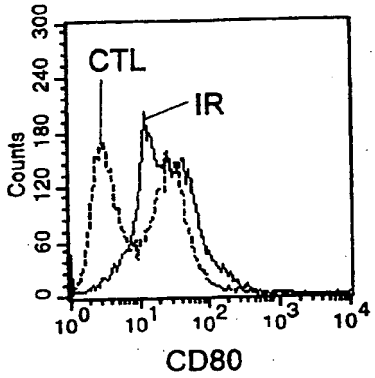

IR-P
CTL
Counts
CD14

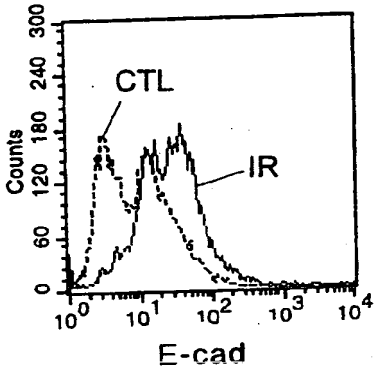
# Figure 30B

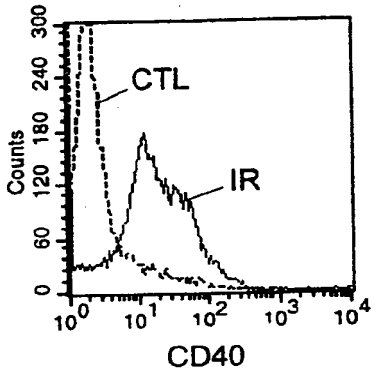


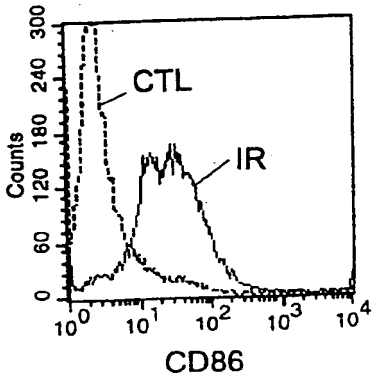
# Figure 31

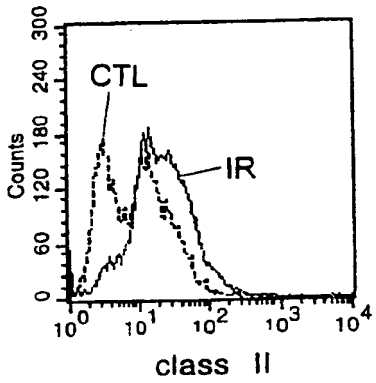

CTL
IR
Counts
CD11c


CTL
IR
Counts
CD80


CTL
IR
Counts
E-cad


CTL
IR
Counts
CD40


CTL
IR
Counts
CD86


CTL
IR
Counts
class II

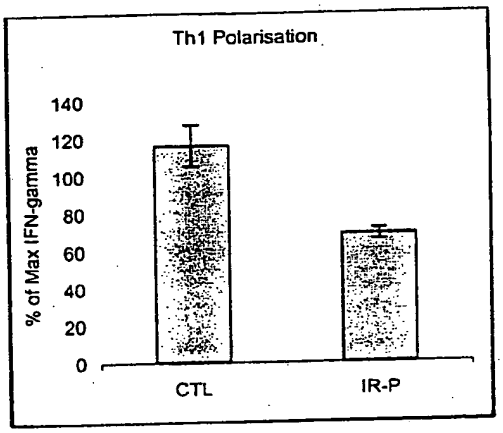



Figure 32

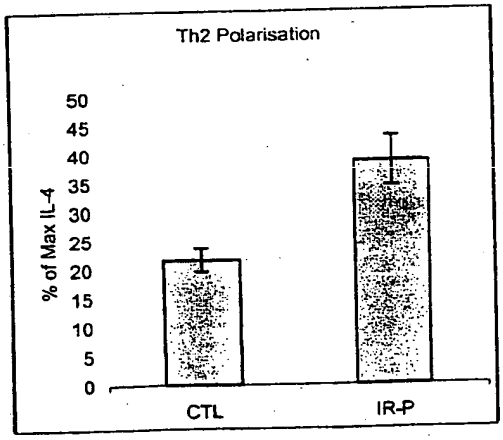



Figure 34

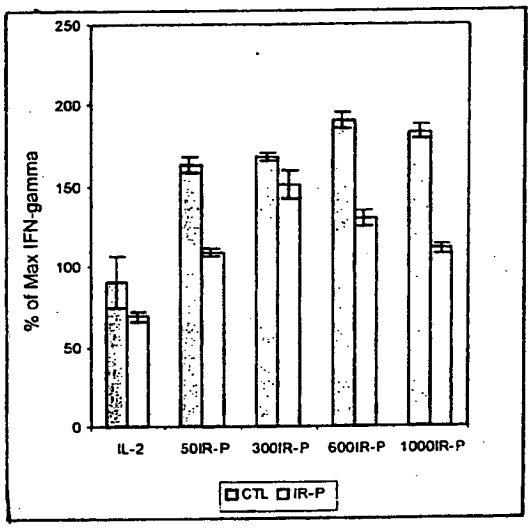

250
200
150
100
50
0
% of Max IFN-gamma
IL-2
50IR-P
300IR-P
600IR-P
1000IR-P
CTL
IR-P

Figure 36

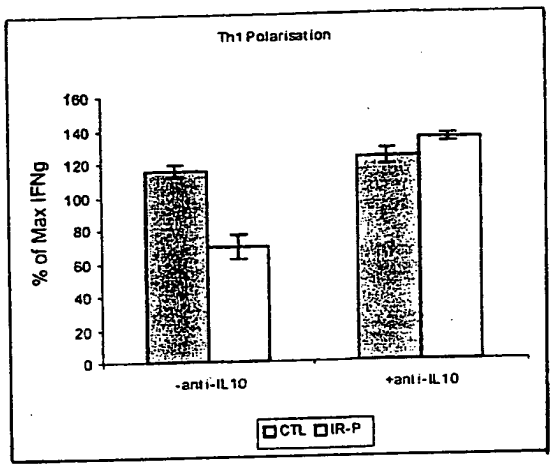



Figure 38

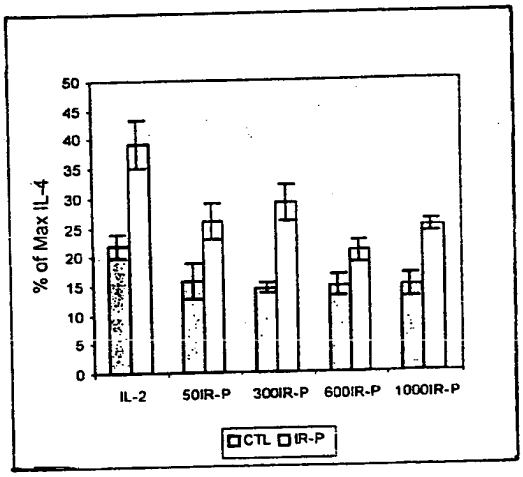



figure 37

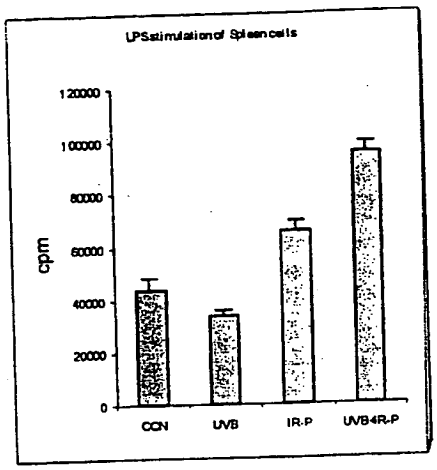



Figure 46.

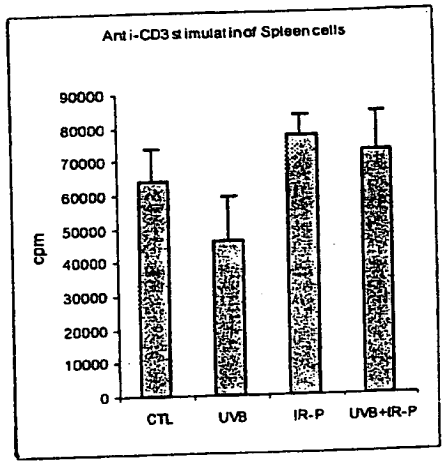



Figure 47.

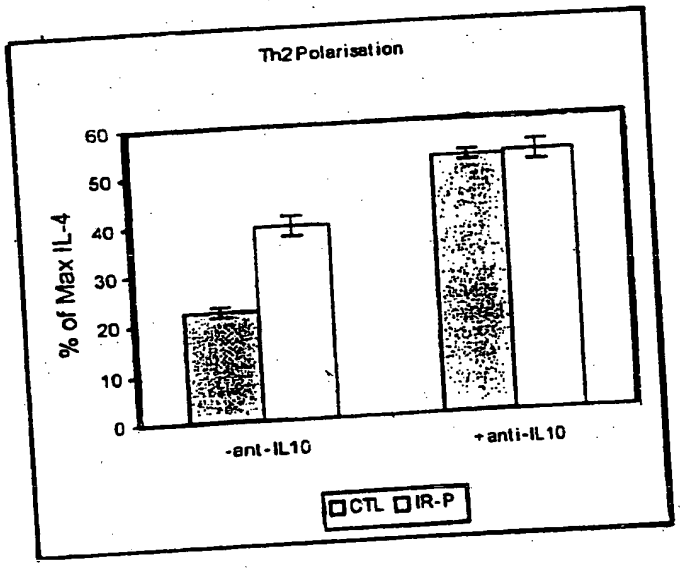

Th2 Polarisation
% of Max IL-4
60
50
40
30
20
10
0
-anti-IL10
+anti-IL10
CTL
IR-P

Figure 39

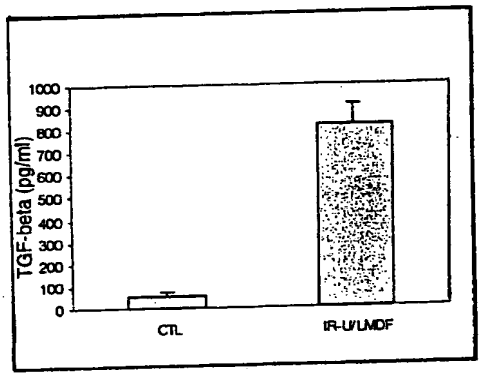



Figure 43

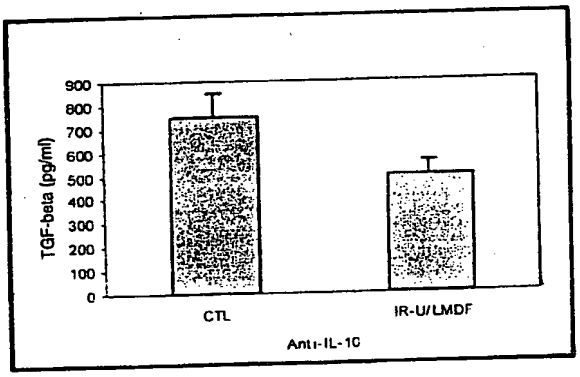



Figure 44 A.

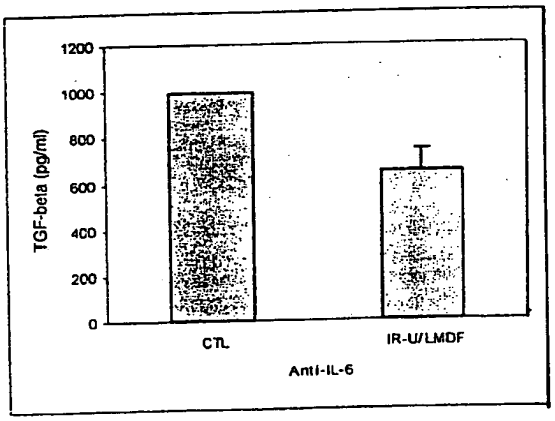



Figure 44 B

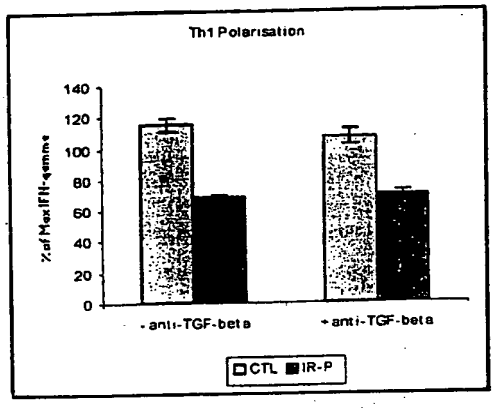



Figure 40.

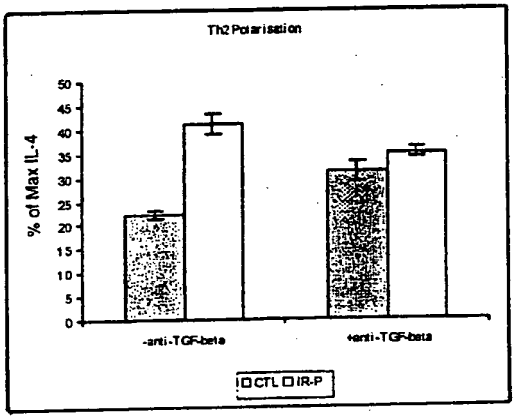



Figure 41

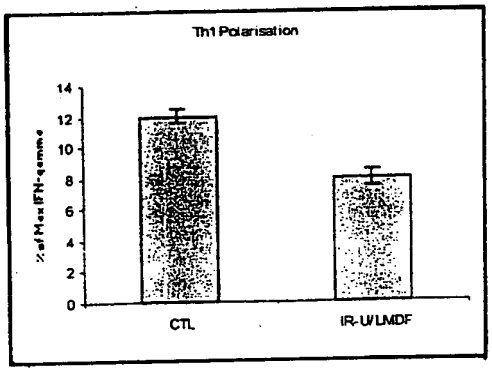



Figure 33.

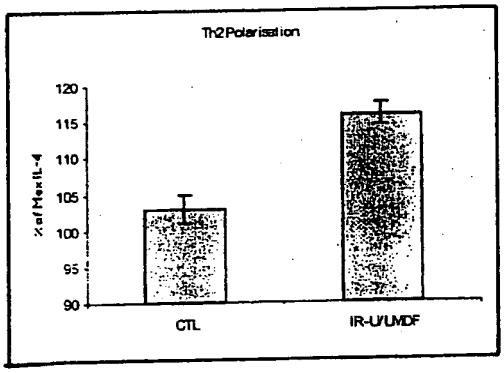



Figure 35.

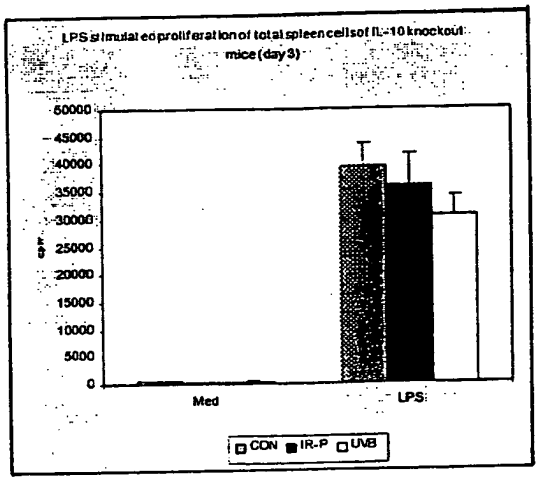



Figure 50

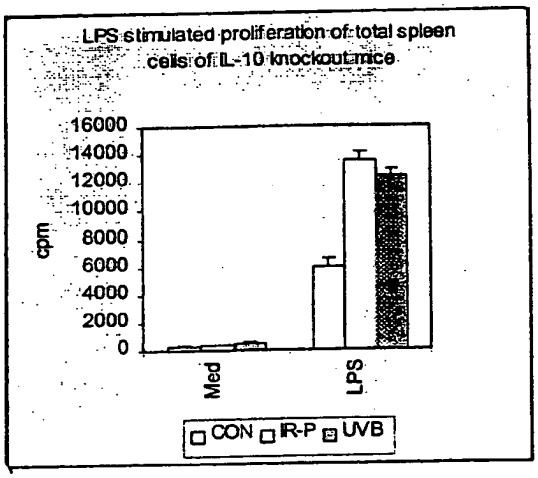



Figure 51

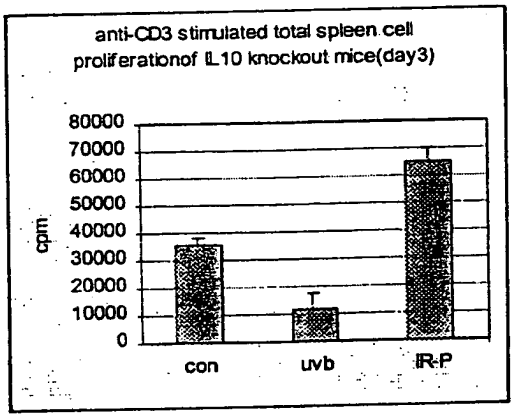



Figure 48

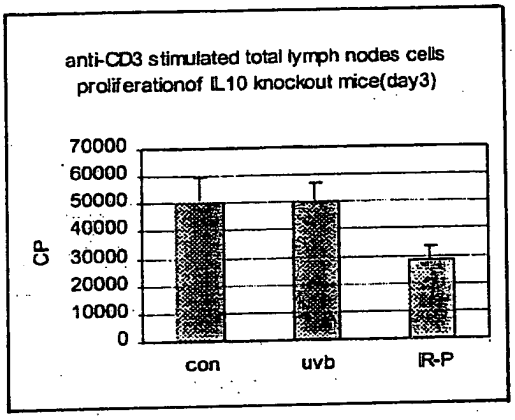



Figure 49

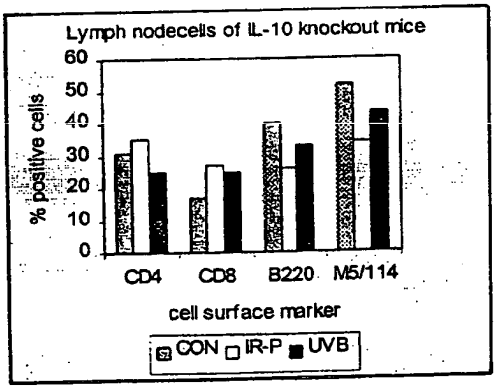



Figure 52

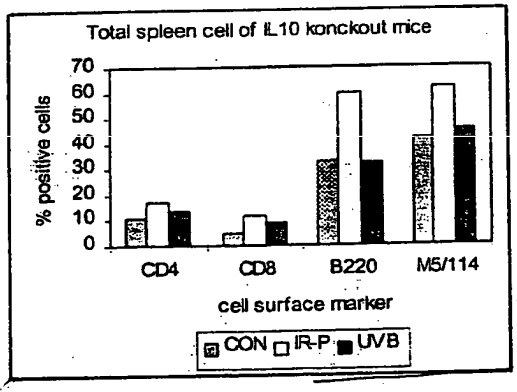



Figure 53

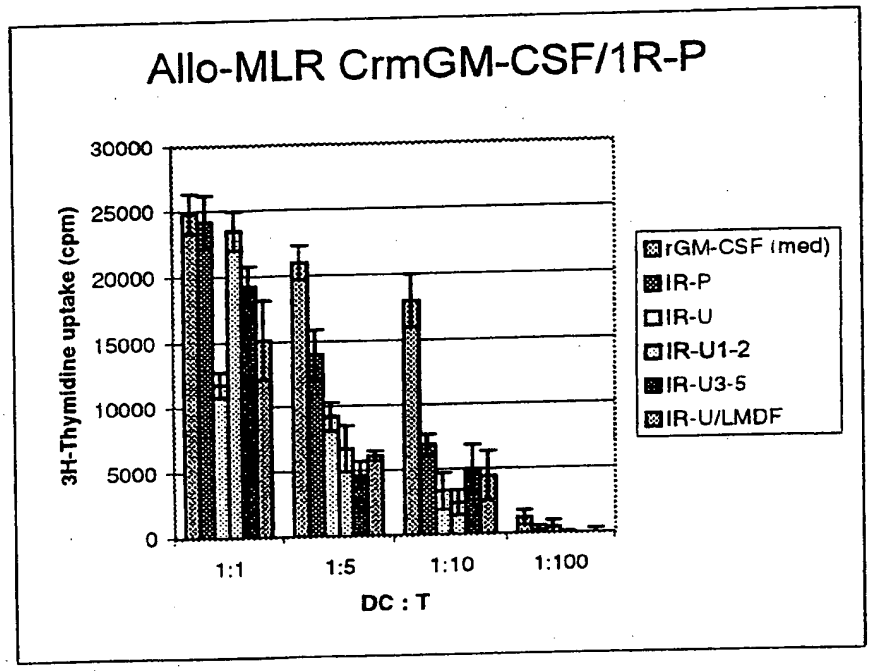
| Mab | Med | IR-P | IR-U | IR-U3-5 | IR-U/LMDF |
|---|---|---|---|---|---|
| CD1d | 4.9 | 3.2 | 2.4 | 2.8 | 2.8 |
| **CD14** | 0.0 | 0.6 | 2.7 | 1.0 | 0.8 |
| CD40 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| CD80 | 0.3 | 0.0 | 0.0 | 0.0 | 0.0 |
| CD86 (all) | 1.9 | 0.8 | 0.1 | 0.5 | 0.6 |
| CD95 (all) | 5.3 | 4.1 | 12.8 | 5.6 | 5.6 |
| CD95L | 0.2 | 0.3 | 0.2 | 0.0 | 0.0 |
| **ER-MP58** | 3.9 | 2.6 | 1.7 | 0.0 | 1.1 |
| **F4/80 (all)** | 39.5 | 20.1 | 1.3 | 2.2 | 0.0 |
| RB6.8C5 | | 3.6 | 5.8 | 5.0 | 4.1 |
| E-cad (all) | 1.9 | 4.5 | 0.5 | 0.5 | 0.9 |
| MHC II | 13.8 | 7.8 | 9.3 | 6.3 | 0.0 |

Figure54

| Mab | Med | IR-P | IR-U | IR-U3-5 | IR-U/LMDF |
|---|---|---|---|---|---|
| **CD1d** | 4.9 | 7.0 | 11.8 | 9.5 | 9.5 |
| CD14 | 0.0 | 1.0 | 0.9 | 1.9 | 1.2 |
| **CD40** | 0.0 | 0.6 | 4.4 | 5.5 | 3.8 |
| **CD80** | 0.3 | 0.3 | 0.9 | 0.7 | 0.6 |
| **CD80 (fractie)** | | | 8.0 (37%) | 16.0 (20%) | 12.8 (20%) |
| **CD86 (all)** | 1.9 | 3.3 | 19.7 | 10 | 11.5 |
| **CD95** | 5.3 | | 15.2 | 16 | 16 |
| ER-MP58 | 3.9 | 5.2 | 6.1 | 7.7 | 7.0 |
| **F4/80 (all)** | 39.5 | 32.2 | 108.8 | 136.9 | 158.7 |
| RB6.8C5 | | 7.7 | 8.2 | 4.0 | 4.3 |
| E-cad (all) | 1.9 | 2.1 | 3.2 | 3.3 | 1.9 |
| **MHC II (all)** | 13.8 | 18.1 | 108.8 | 94.5 | 109.6 |

Figure 55

Figure 56


Allo-MLR CrmGM-CSF/1R-P
3H-Thymidine uptake (cpm)
30000
25000
20000
15000
10000
5000
0
1:1
1:5
1:10
1:100
DC : T
rGM-CSF (med)
IR-P
IR-U
IR-U1-2
IR-U3-5
IR-U/LMDF

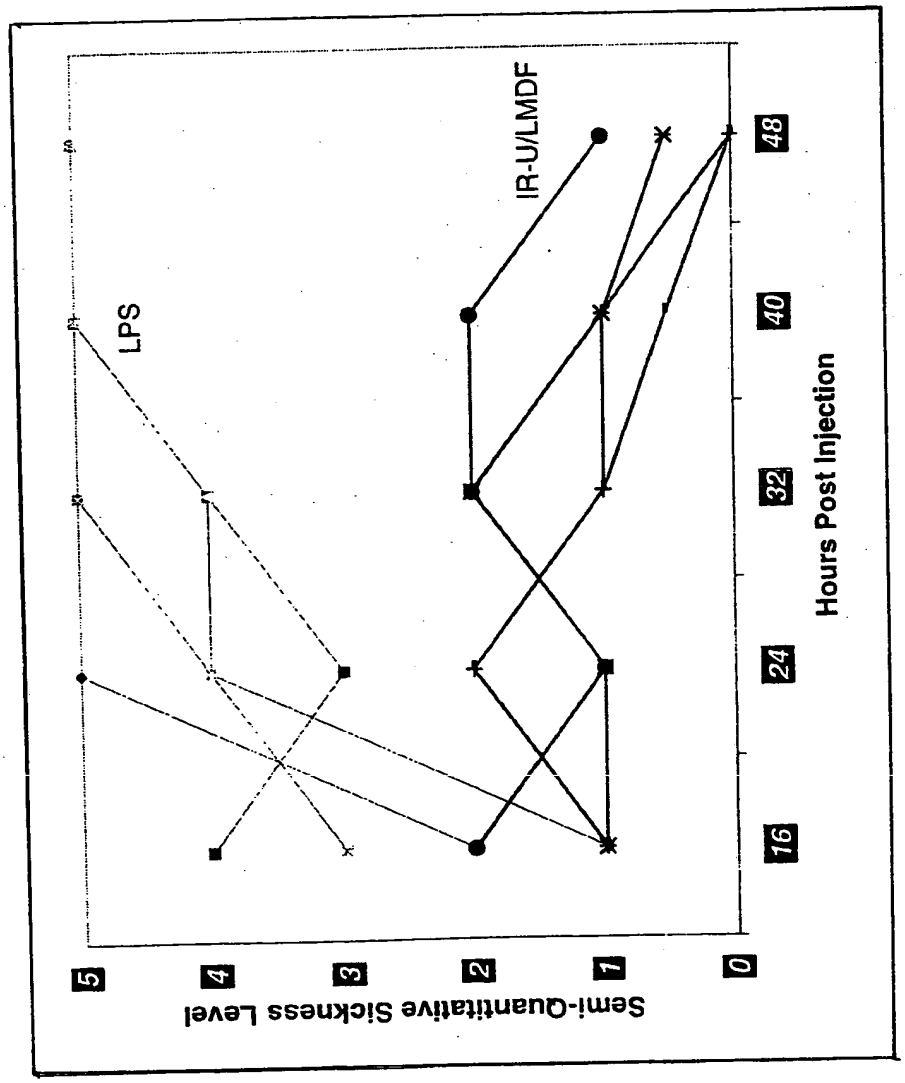

Semi-Quantitative Sickness Level
5
4
3
2
1
0
Hours Post Injection
16
24
32
40
48
LPS
IR-U/LMDF

Figure 57

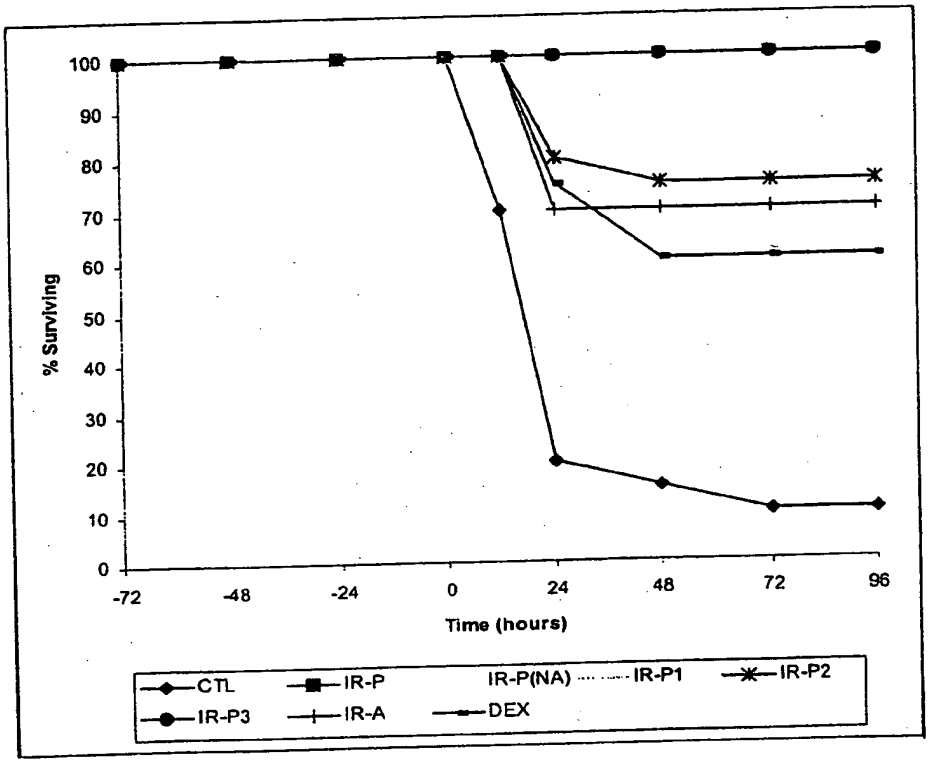

100
90
80
70
60
50
40
30
20
10
0
% Surviving
-72
-48
-24
0
24
48
72
96
Time (hours)
CTL
IR-P
IR-P(NA)
IR-P1
IR-P2
IR-P3
IR-A
DEX

Figure 58

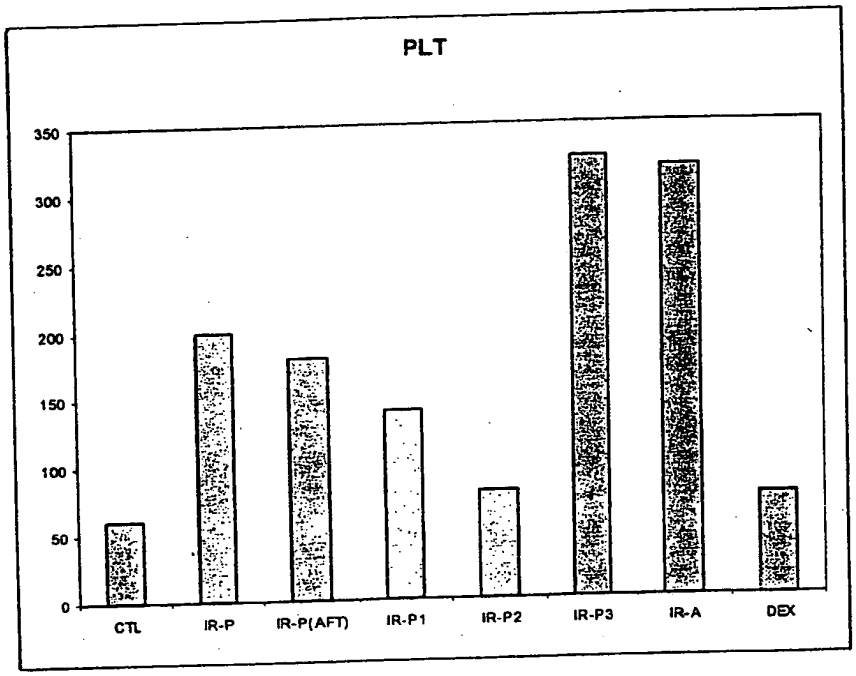

PLT
350
300
250
200
150
100
50
0
CTL
IR-P
IR-P(AFT)
IR-P1
IR-P2
IR-P3
IR-A
DEX

Figure 59

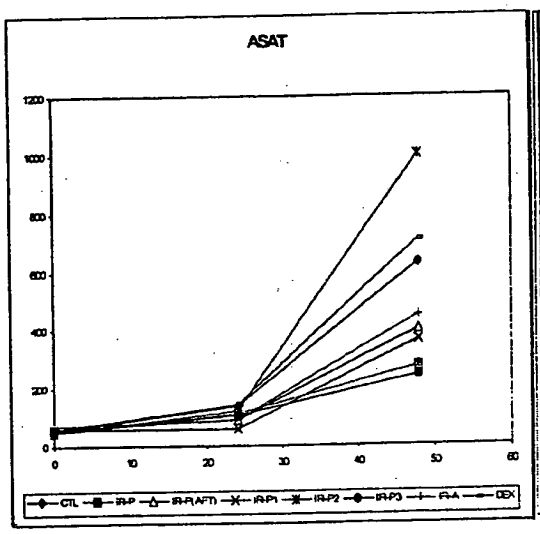



Figure 61

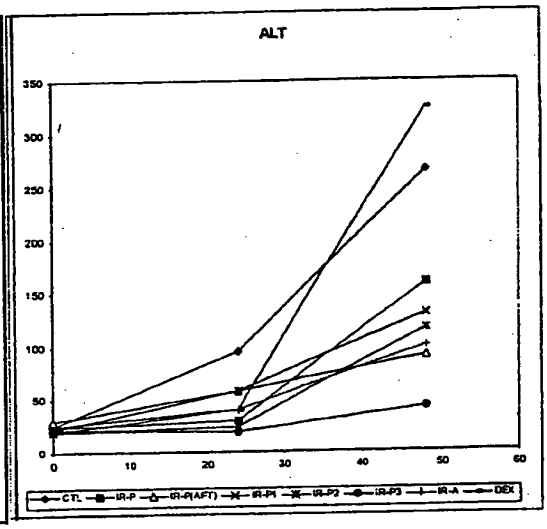



Figure 60

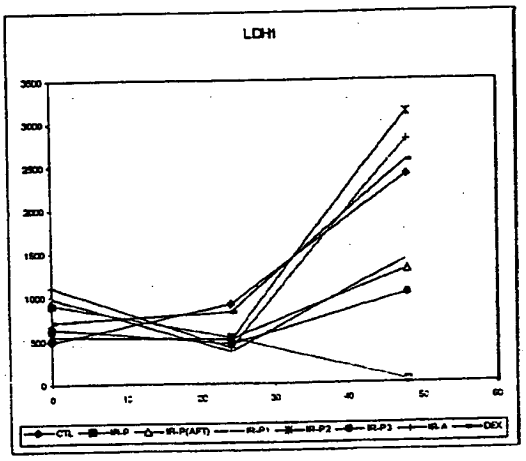



Figure 62

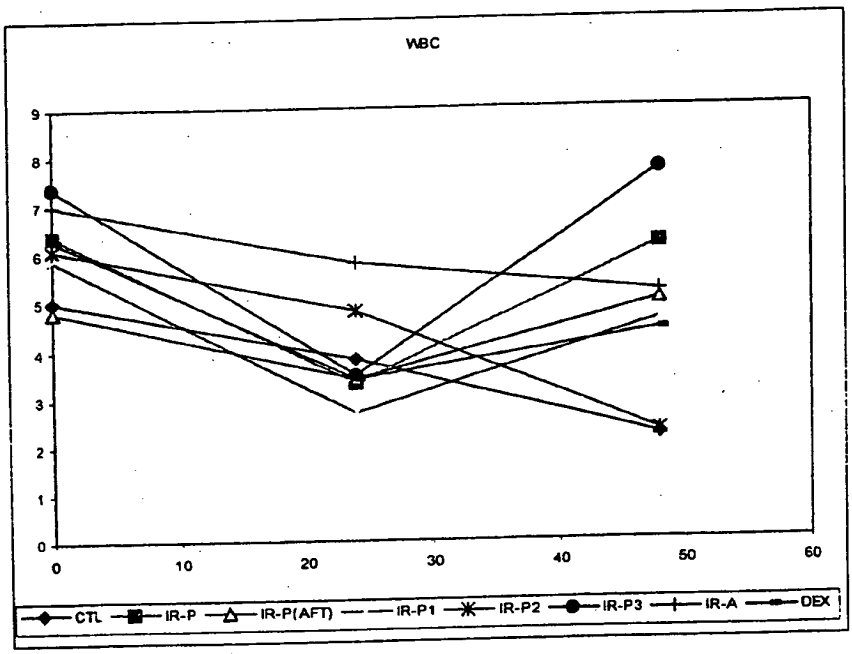

WBC
9
8
7
6
5
4
3
2
1
0
0
10
20
30
40
50
60
CTL
IR-P
IR-P(AFT)
IR-P1
IR-P2
IR-P3
IR-A
DEX

Figure 63

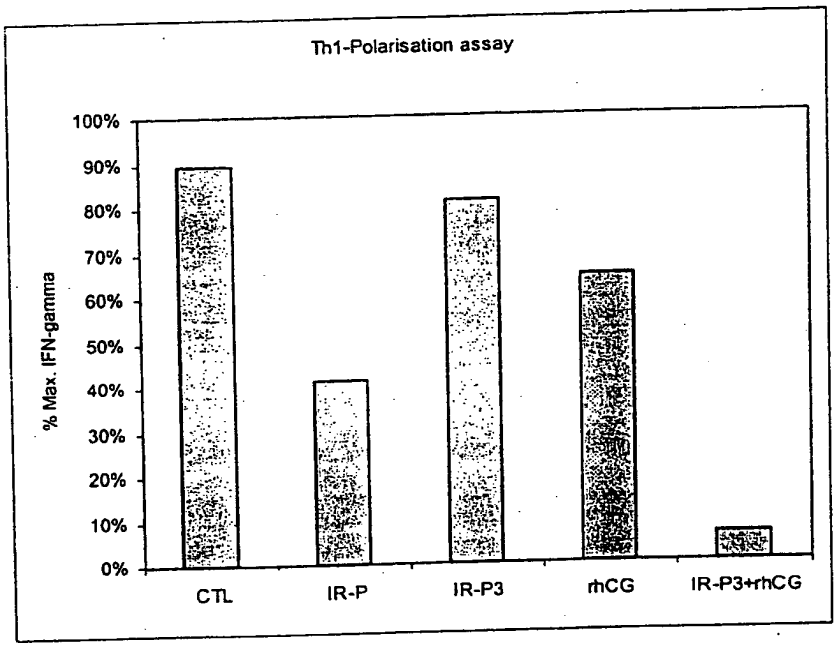

Th1-Polarisation assay
% Max. IFN-gamma
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
CTL
IR-P
IR-P3
rhCG
IR-P3+rhCG

Figure 64

NOD/LTJ INVIVO TREATMENT (ANTI-CD3 STIMULATION)

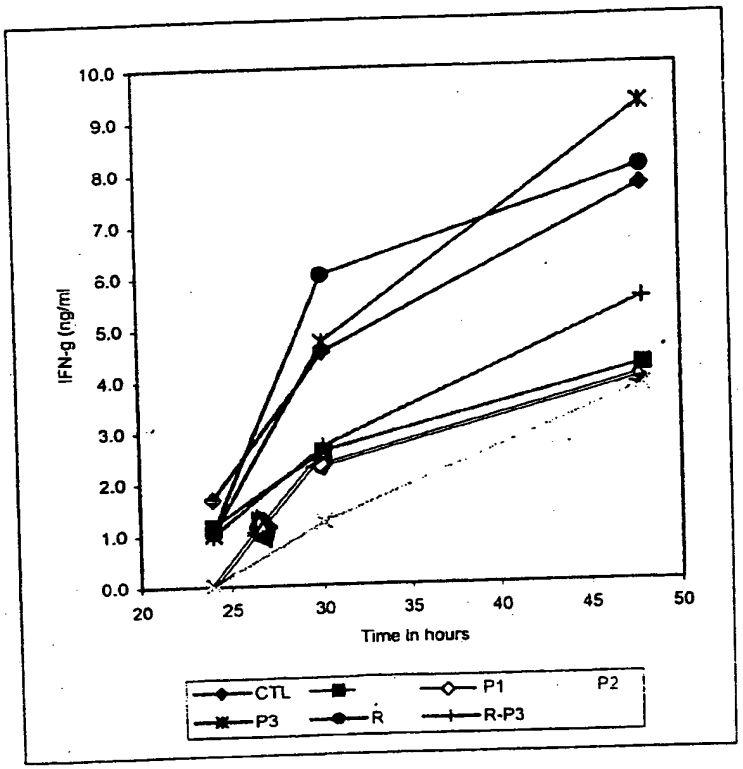



Figure 65

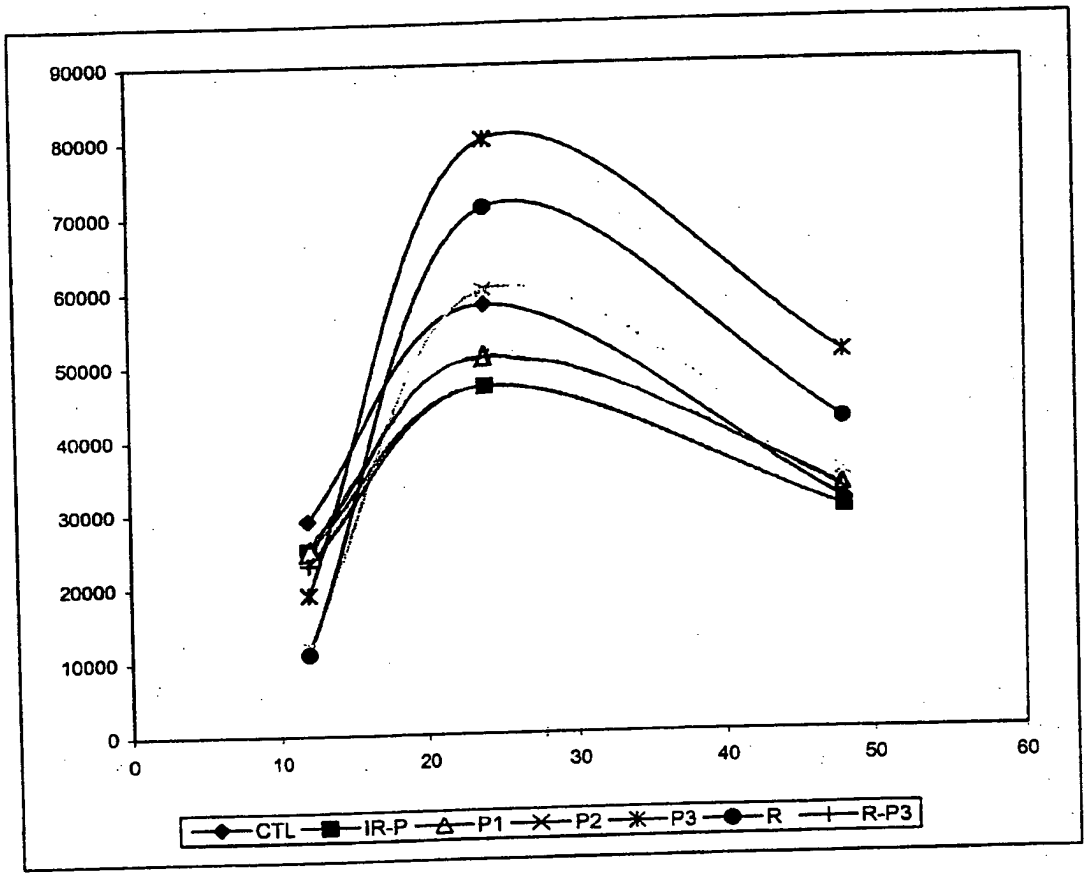

90000
80000
70000
60000
50000
40000
30000
20000
10000
0
0
10
20
30
40
50
60
CTL
IR-P
P1
P2
P3
R
R-P3

Figure 66

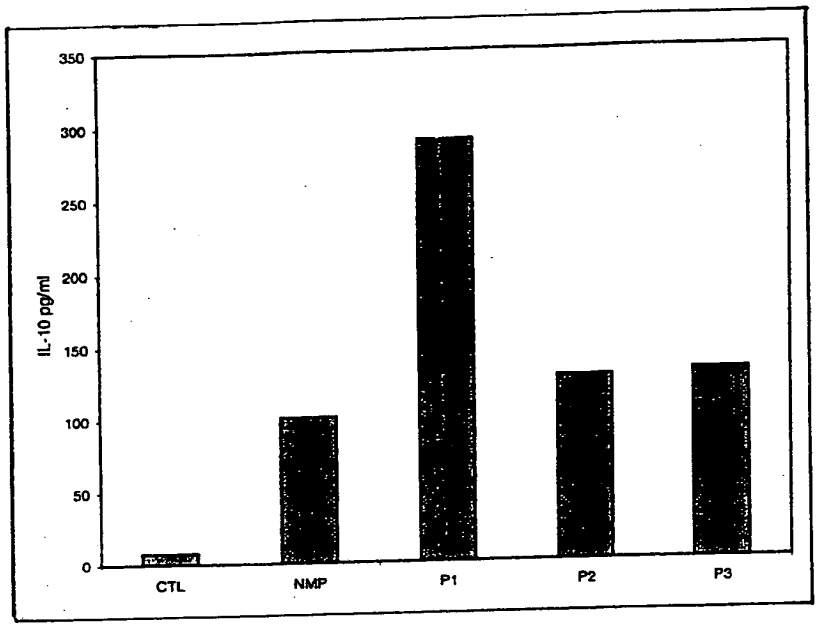



Figure 67

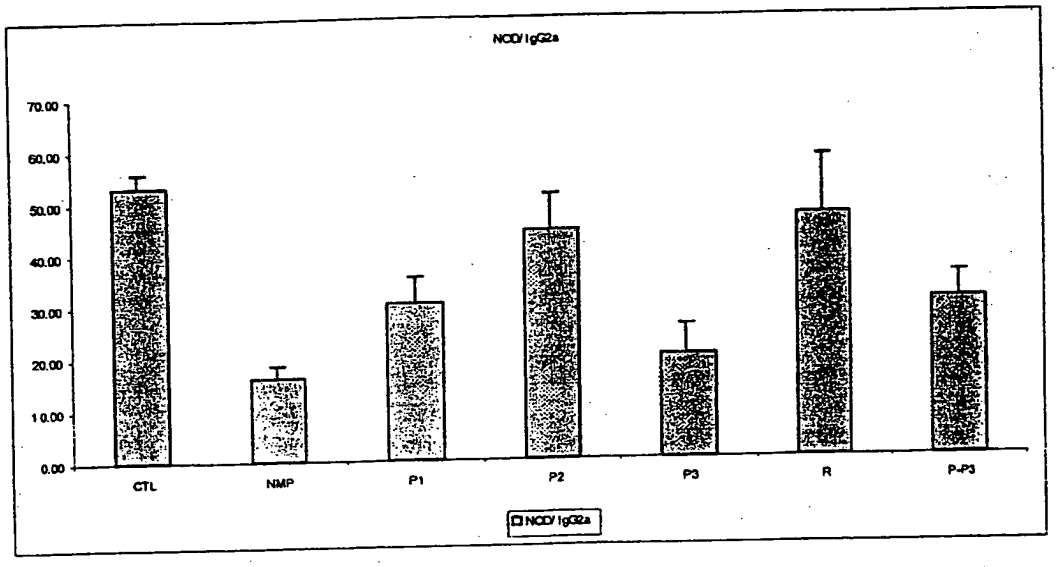

NOD/IgG2a
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
CTL
NMP
P1
P2
P3
R
P-P3
NOD/IgG2a

Figure 68

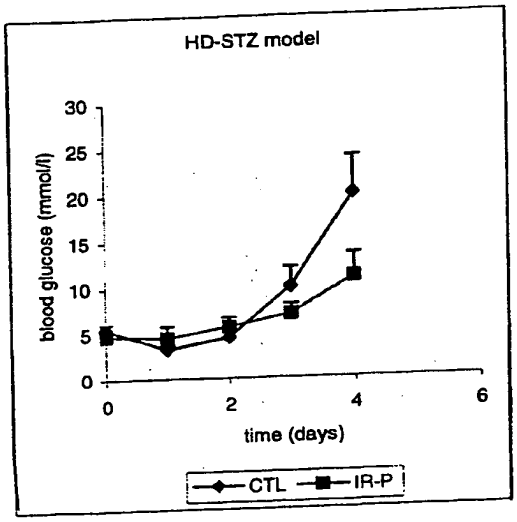



Figure 70

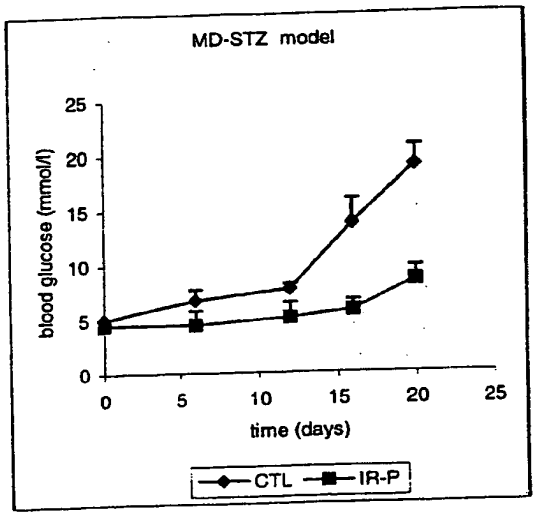



Figure 69

Figure 71

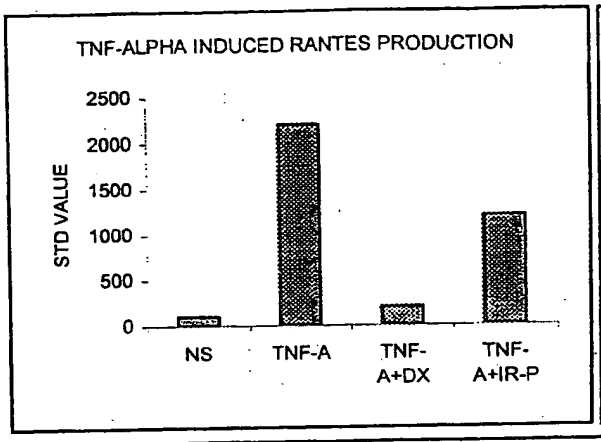
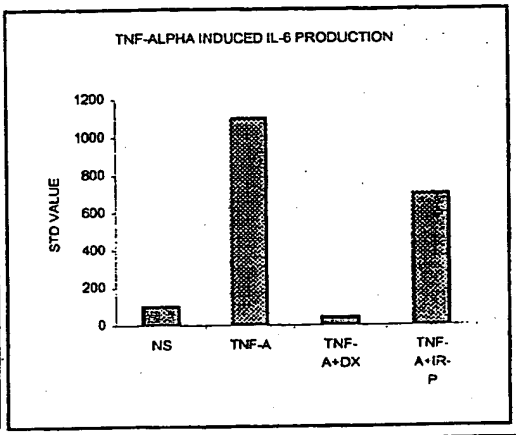



Figure 72



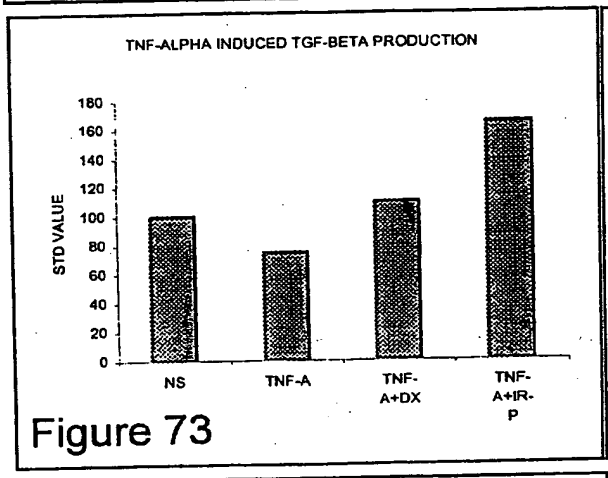




Figure 73

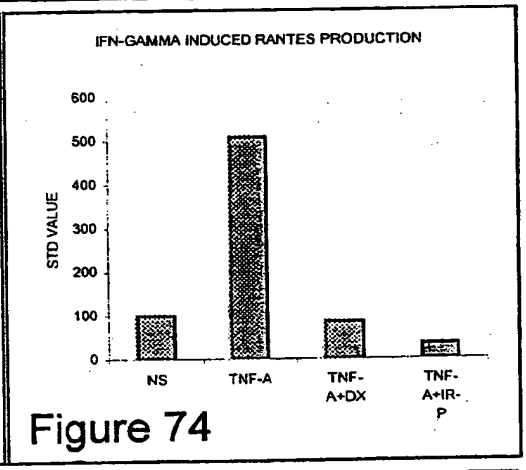



Figure 74

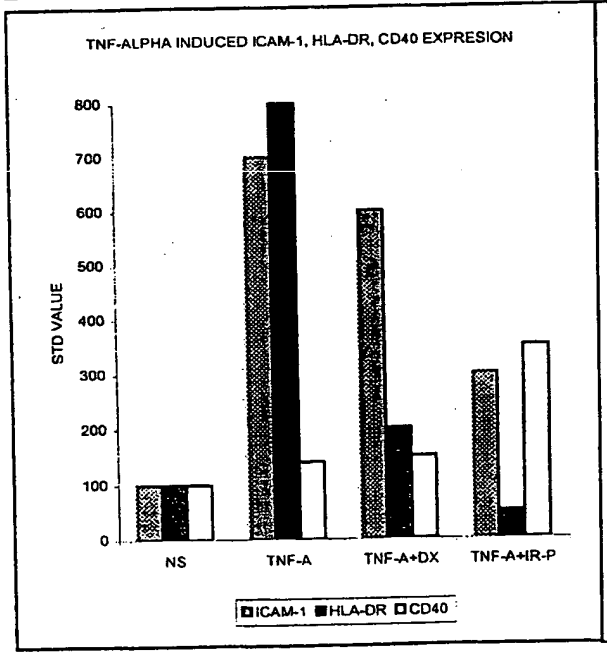



Figure 75

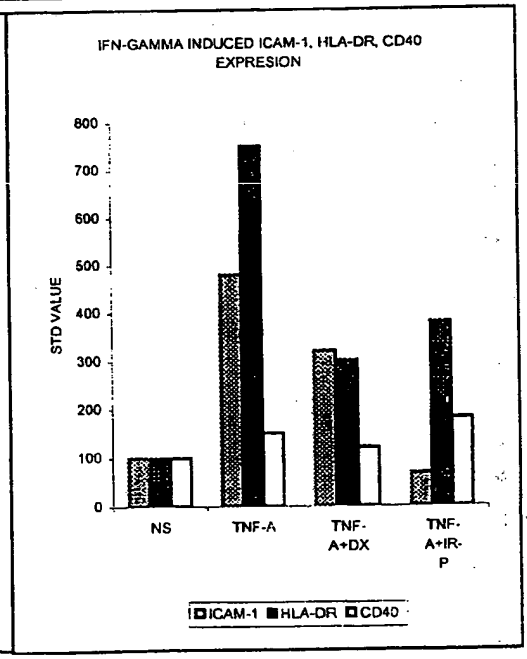



Figure 76

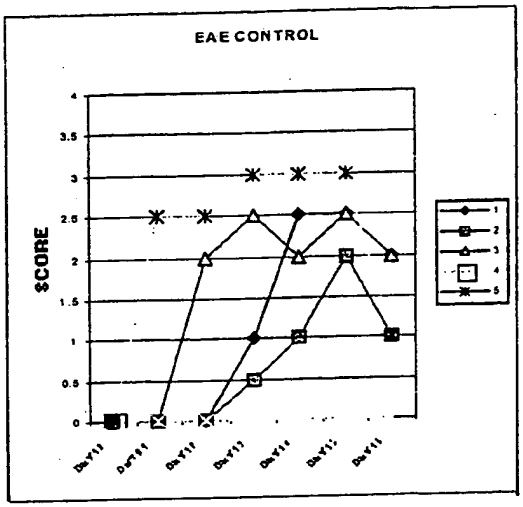



Figure 77

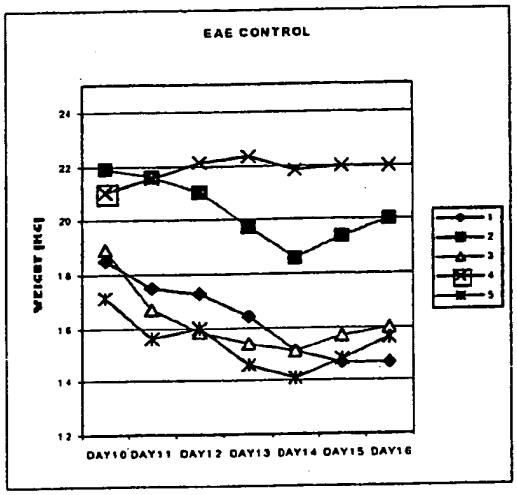



figure 78

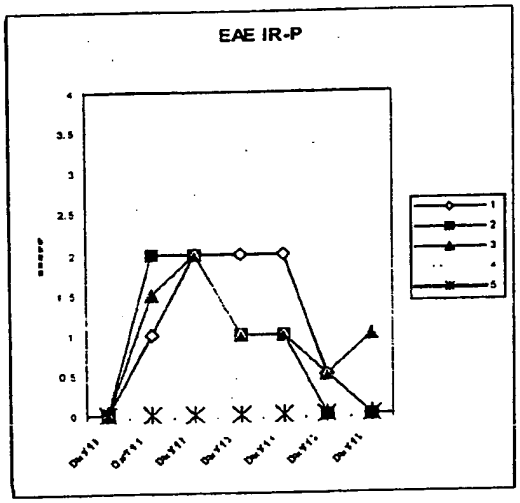



Figure 79

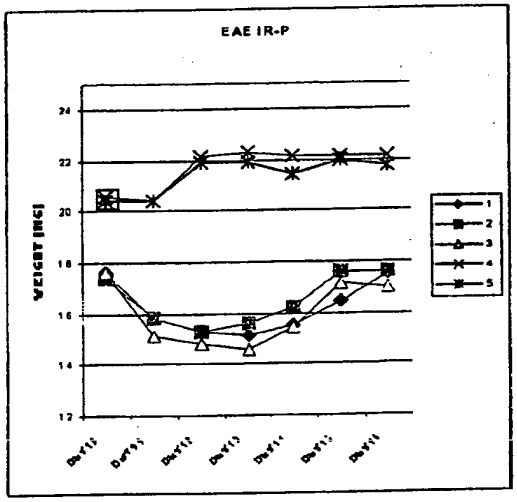
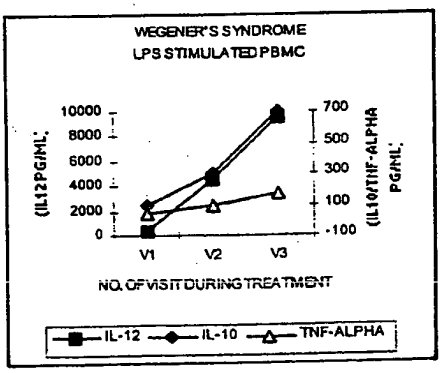



Figure 80

Figure 81

| | Before Tx | during Tx | end Tx | Normal (X 10e9 |
|---|---|---|---|---|
| Lymphocytes | 0.59 | 0.75 | 1.56 | 1.5 - 4.0 |
| T cell | 0.57 | 0.72 | 1.48 | 0.9 - 2.8 |
| CD4 | 0.24 | 0.26 | 0.59 | 0.5 - 1.7 |
| CD8 | 0.31 | 0.41 | 0.23 | 0.3 - 0.8 |
| B-cell | 0.01 | 0.01 | 0.01 | 0.1 - 0.3 |

Figure (82a)




(82b)

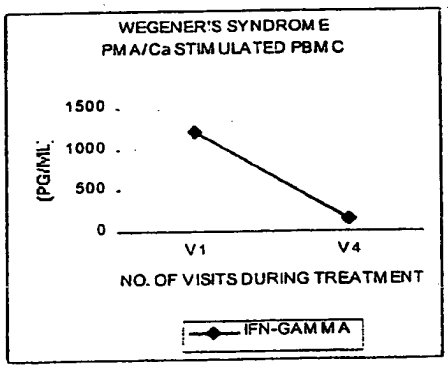


Figure 83

| | Before Tx | during Tx | end Tx | Normal (X 10e9 |
|---|---|---|---|---|
| Lymphocytes | 2.87 | 2.06 | 1.22 | 1.5 - 4.0 |
| T cell | 2.35 | 1.59 | 1.02 | 0.9 - 2.8 |
| CD4 | 1.95 | 1.26 | 0.82 | 0.5 - 1.7 |
| CD8 | 0.49 | 0.37 | 0.18 | 0.3 - 0.8 |
| B-cell | 0.33 | 0.19 | 0.14 | 0.1 - 0.3 |

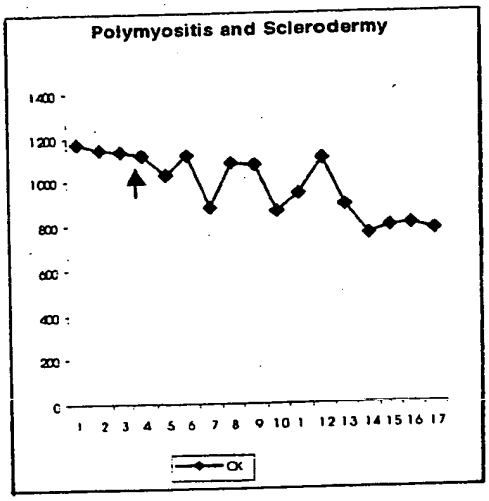



Figure 84

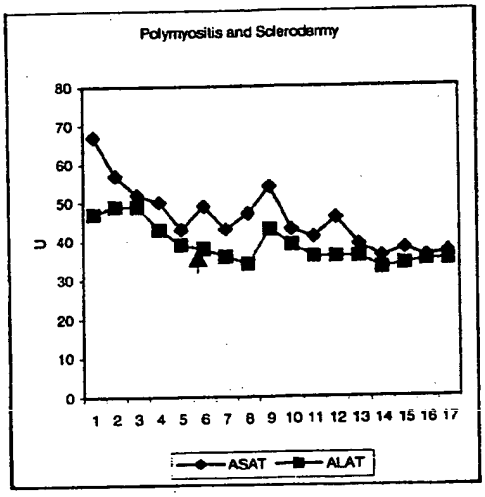



Figure 85

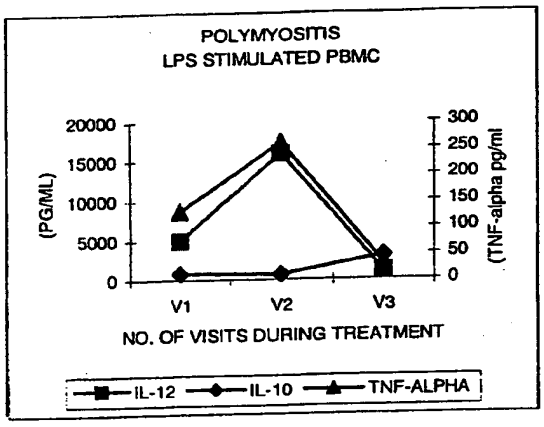

POLYMYOSITIS
LPS STIMULATED PBMC
20000
15000
10000
5000
0
(PG/ML)
300
250
200
150
100
50
0
(TNF-alpha pg/ml
V1
V2
V3
NO. OF VISITS DURING TREATMENT
IL-12
IL-10
TNF-ALPHA

Figure 86

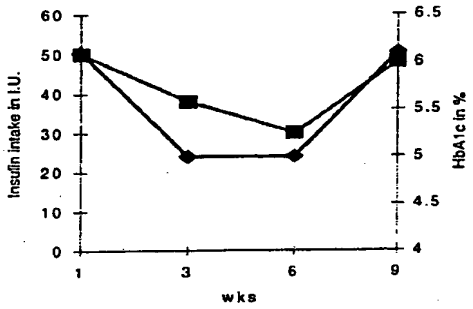



Figure 87

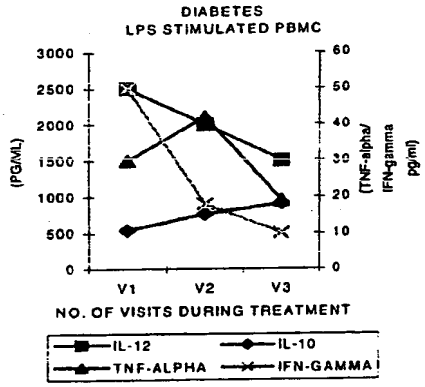



Figure 88

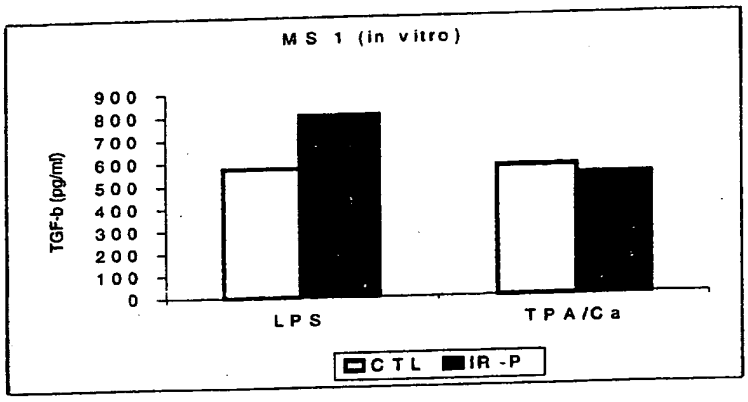



Figure 89

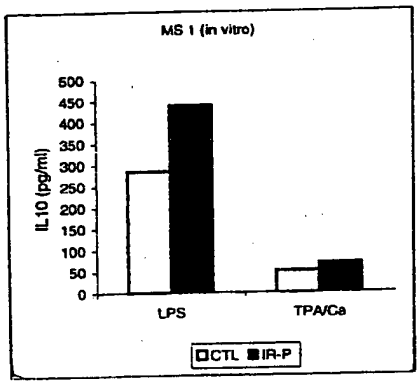



Figure 90

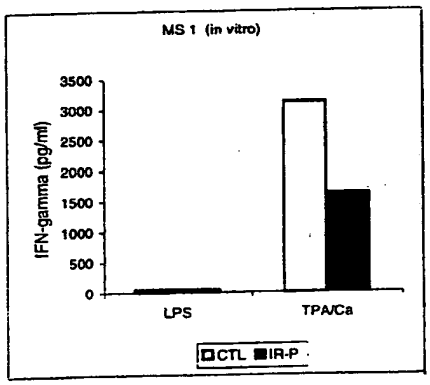



Figure 91

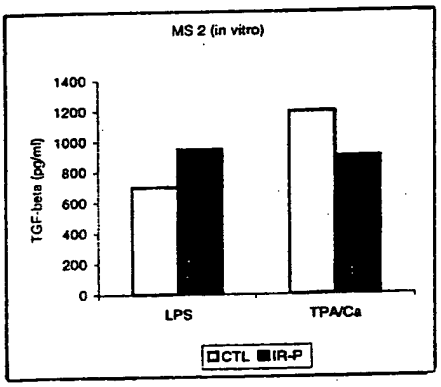



Figure 92

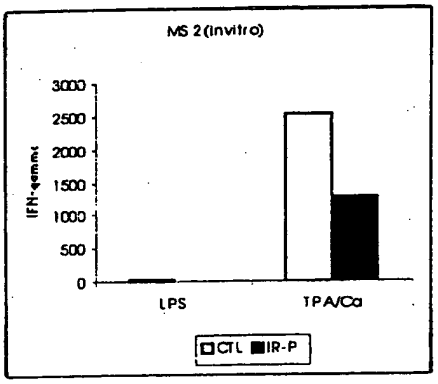



Figure 93

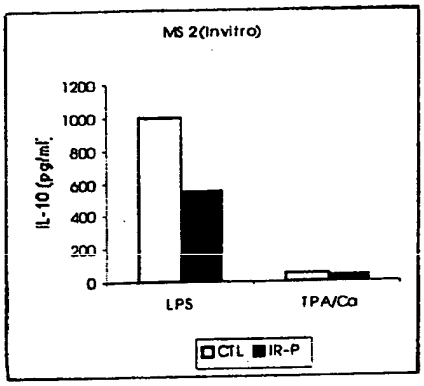



Figure 94

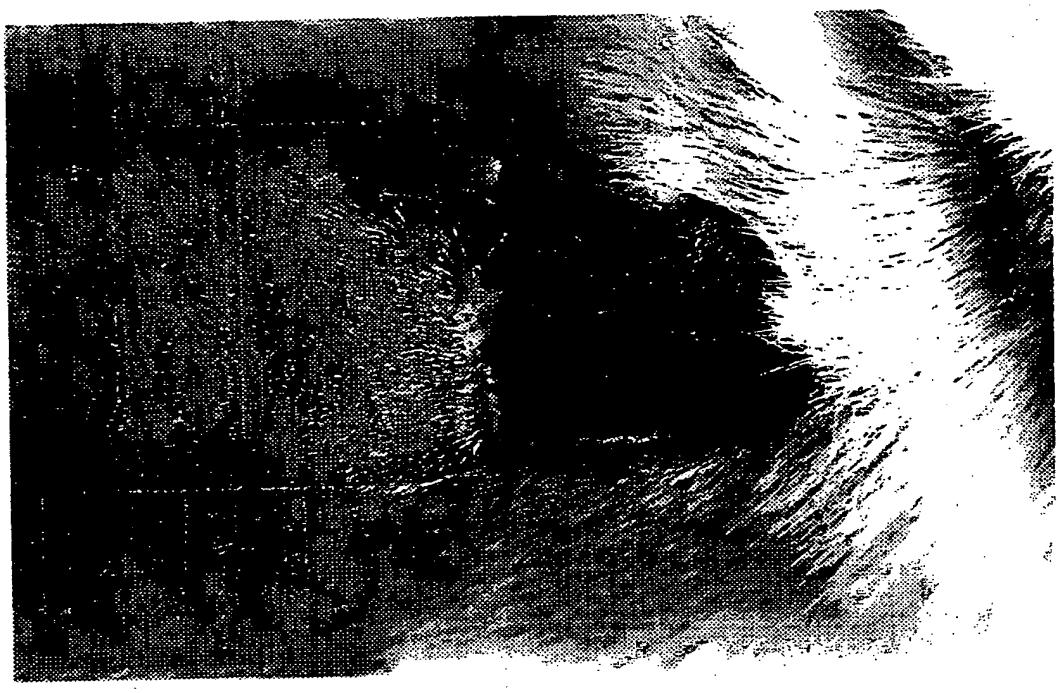


Figure 96

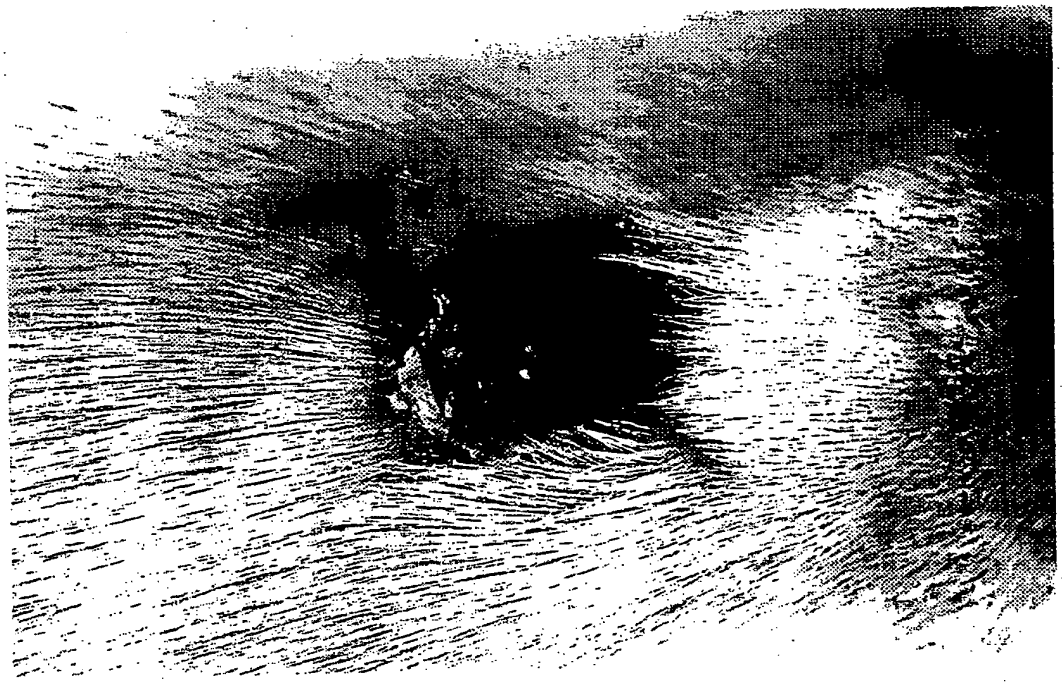


Figure 95

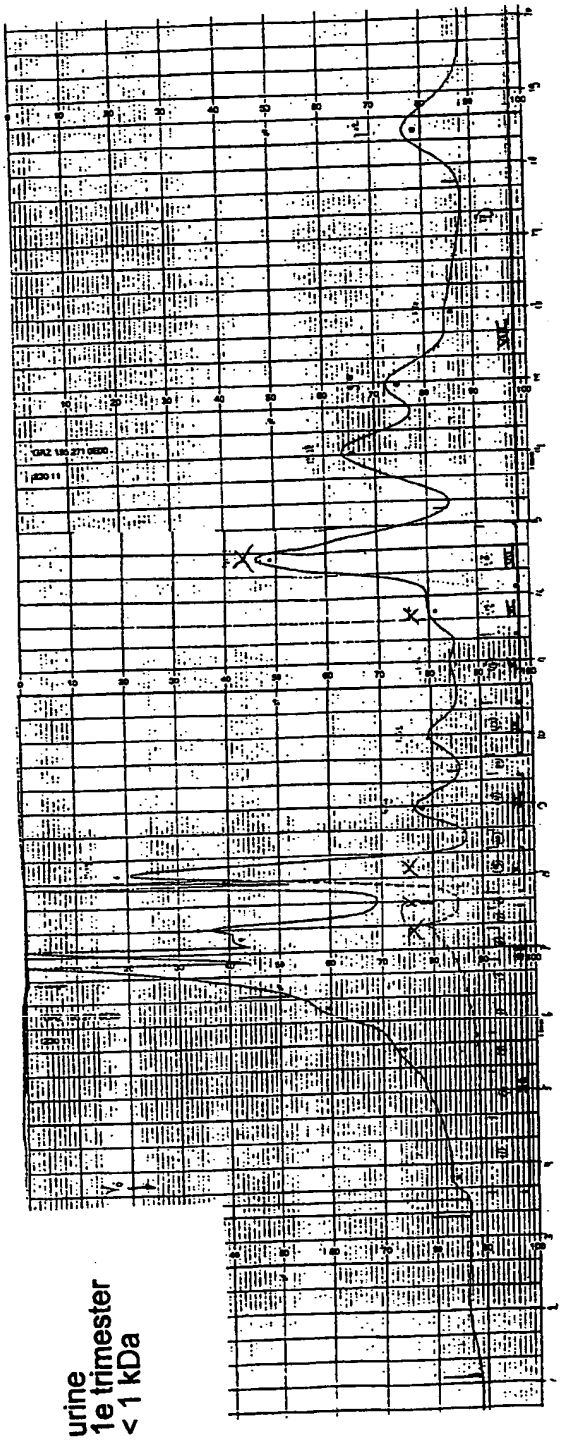



Figure 97

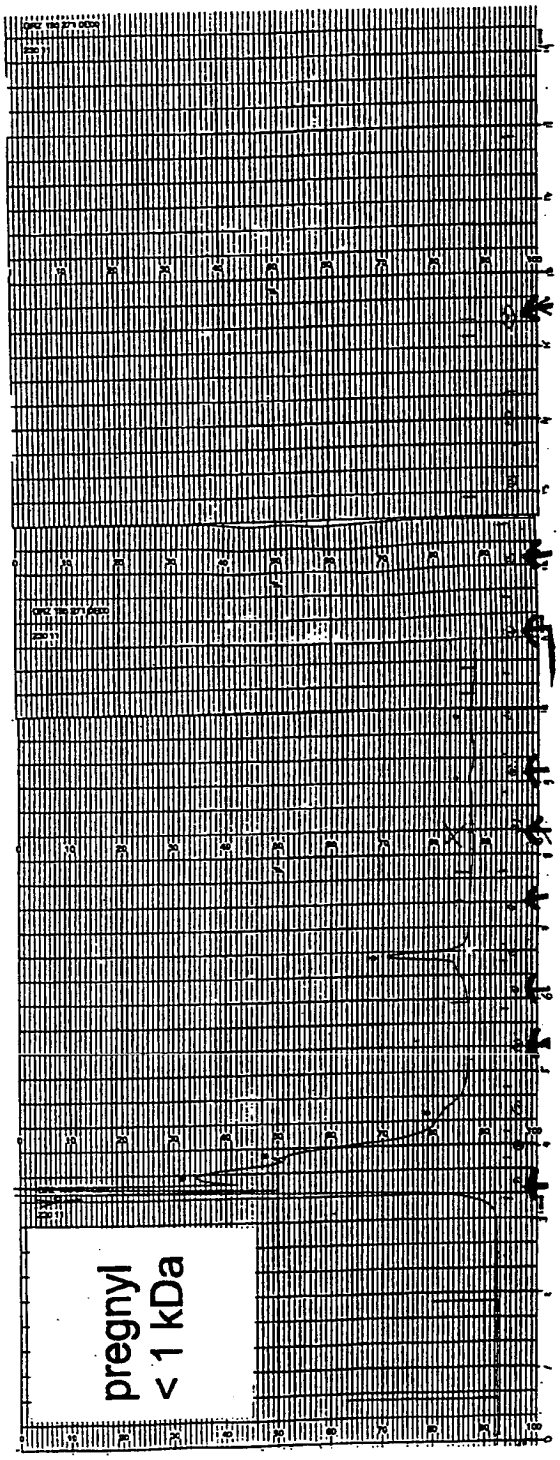



Figure 98

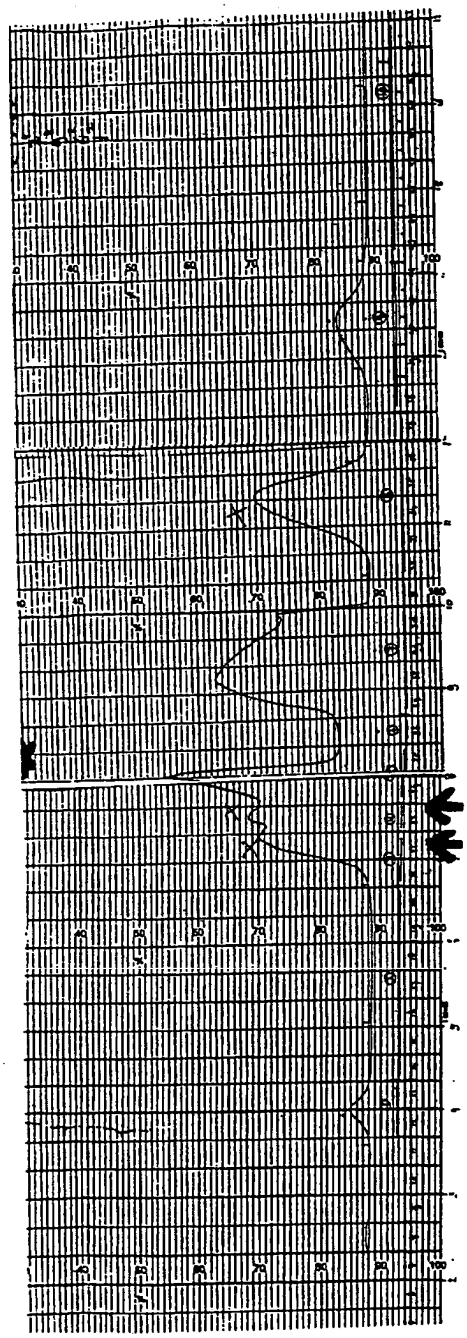


Figure 99

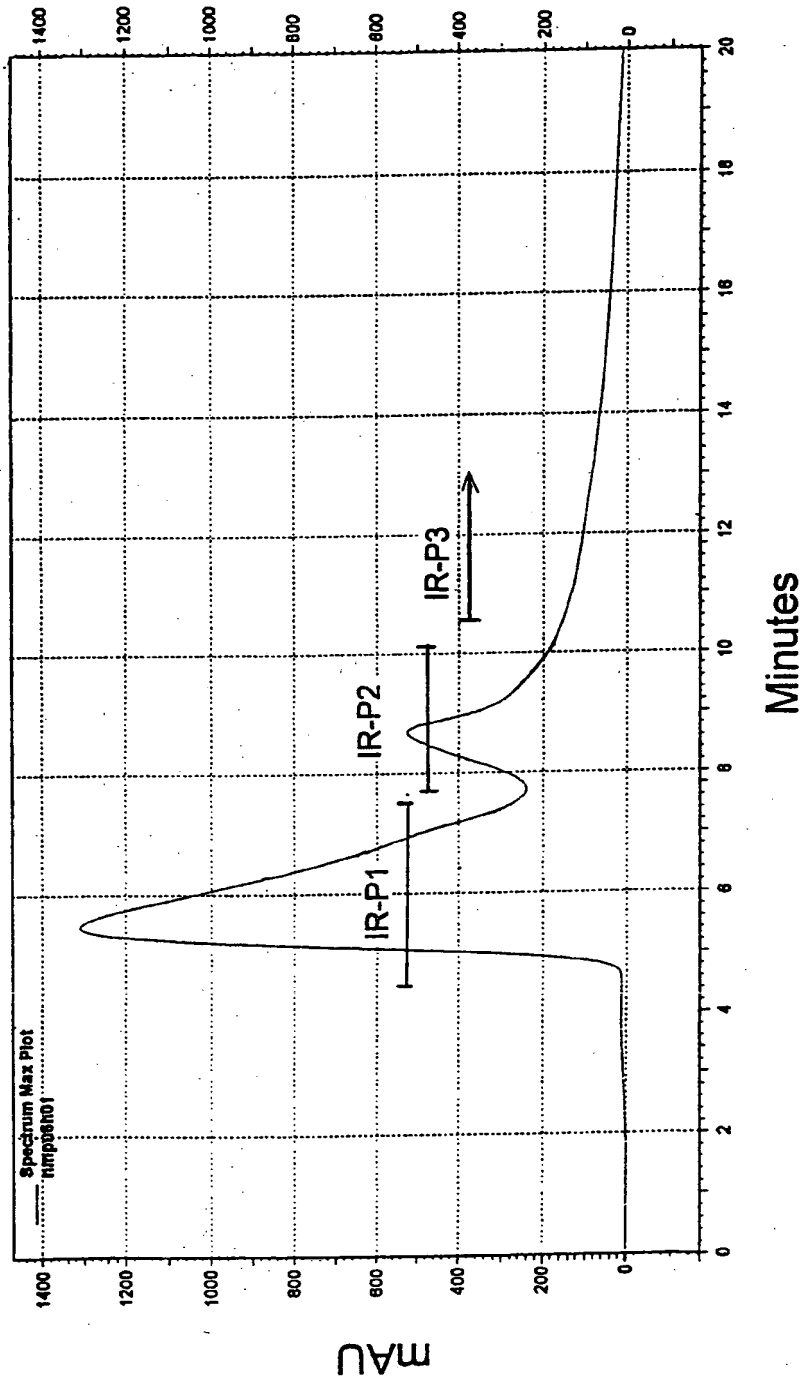
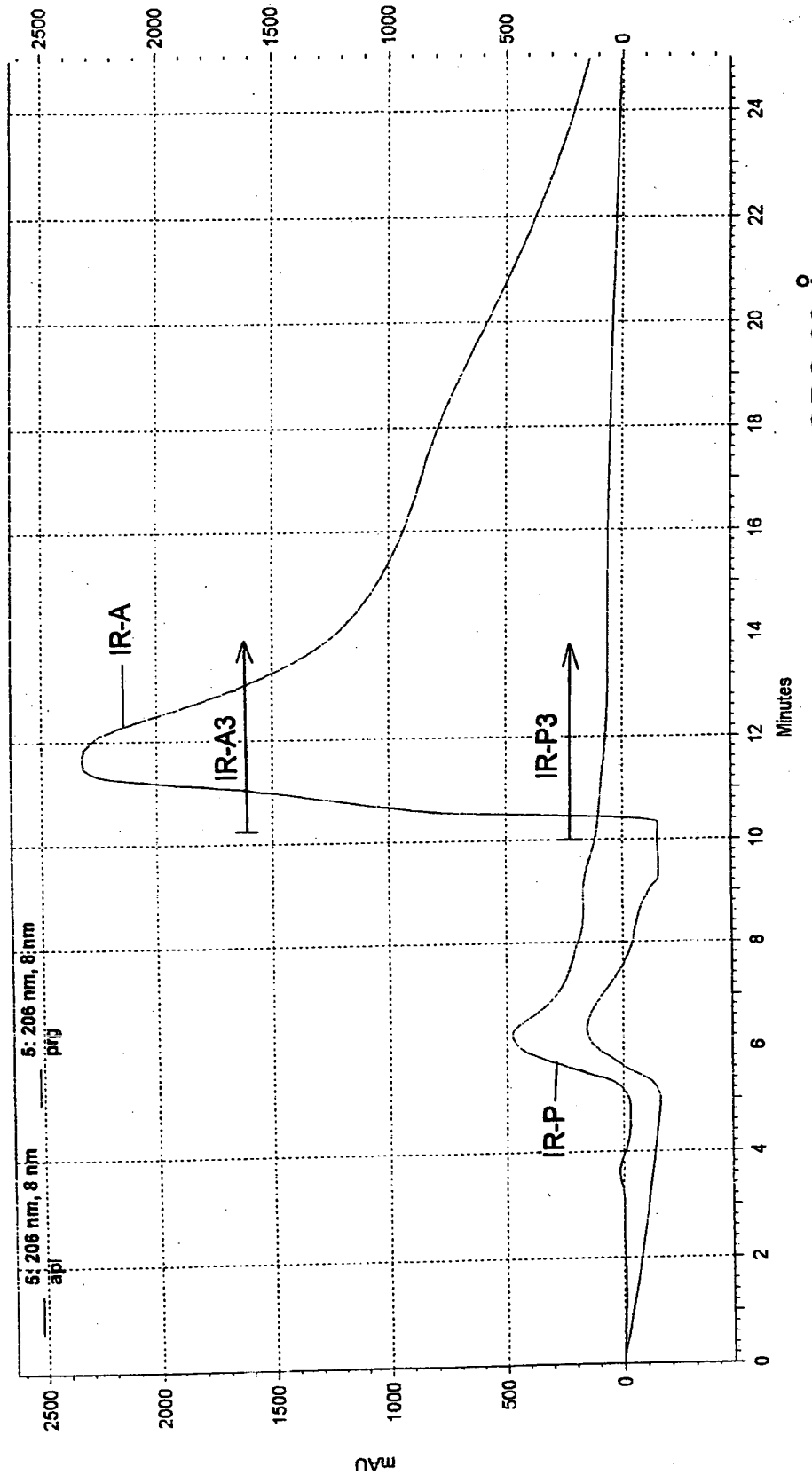



Figure 100




GPC 60 Å

Figure 101

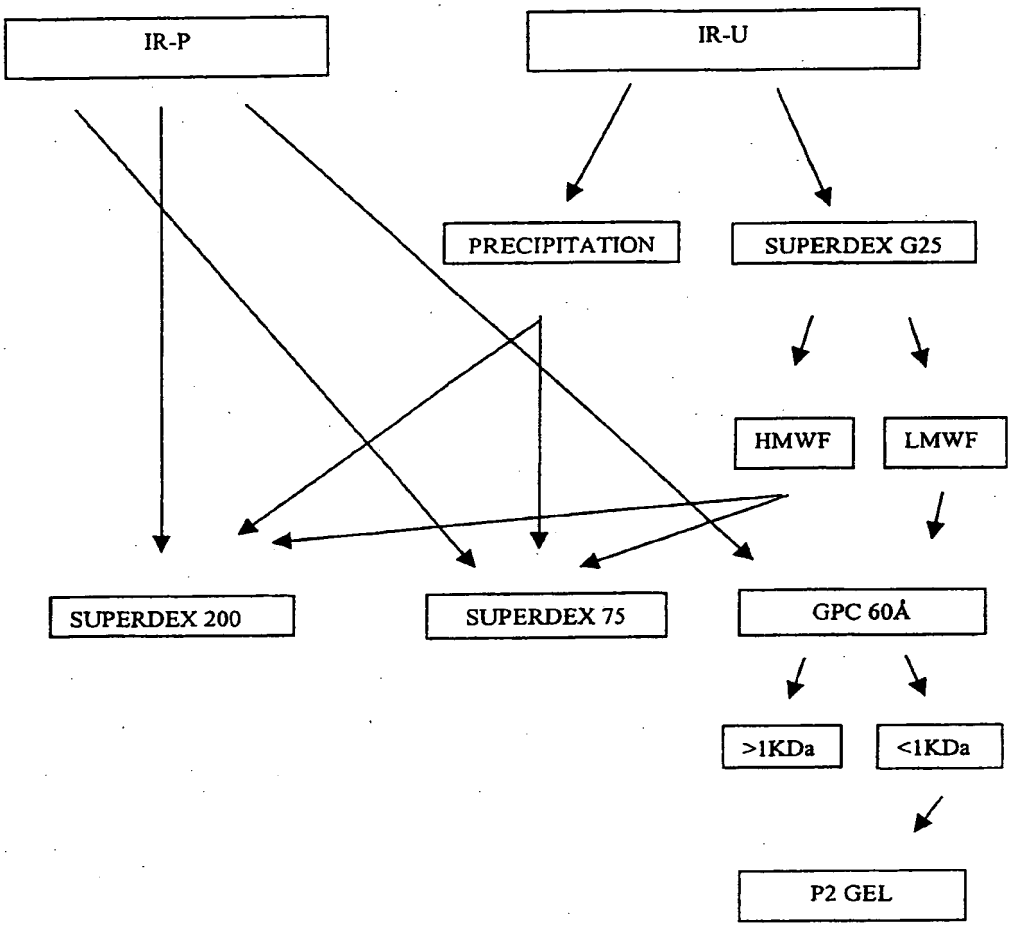

IR-P
IR-U
PRECIPITATION
SUPERDEX G25
HMWF
LMWF
SUPERDEX 200
SUPERDEX 75
GPC 60Å
>1KDa
<1KDa
P2 GEL

Figure 102